PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 27 JULY, 1960 NUMBER 2

## *Eufilaria mcintoshi*, n.sp. from *Padda oryzivora* (L)

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A new species of *Eufilaria* Seurat, 1921 has been found in a Java sparrow purchased from a wholesale pet dealer in St. Thomas, Ontario. This species is named in honour of Dr. Allen McIntosh of the Agricultural Research Service, Beltsville.

DESCRIPTION (Figs. 1-8): Filarioidea, Onchocercidae, Eufilariinae, *Eufilaria* Seurat, 1921. Small, slender, with extremities only slightly attenuated. Buccal cavity absent, oral opening minute. Amphids large, displaced slightly to ventral side. Cephalic papillae consisting of four papillae on dorsal side of cephalic extremity. Oesophagus short, narrow, undivided, indistinctly separated from intestine, the latter full of erythrocytes (?) and constricted at various points. Excretory pore slightly behind nerve ring. Phasmids much reduced, terminal in females, not observed in male. Deirids absent. Lateral fields broad and clear. Cuticle delicate, with minute, regular, transverse striations.

MALE (holotype): Length 6.2 mm. Maximum width 71 microns, near middle of body. Width of cephalic end 48 microns. Oesophagus 186 microns in length. Nerve ring 92 microns from cephalic extremity. Anus subterminal, 14 microns from caudal extremity, guarded by pair of lateral, fleshy elevations and single preanal swelling. Spicules subequal, dissimilar morphologically, distal half tapering markedly; right spicule 52 microns, left spicule 64 microns in length.

FEMALE (two specimens; first figure allotype): Length 17.5, 15.5 mm. Maximum width 150, 143 microns, near middle of body. Width cephalic extremity 80, 123 microns. Oesophagus 232, 185 microns in length. Nerve ring 162, 170 microns, excretory pore 185, 190 microns and vulva 450, 460 microns from cephalic extremity. Vagina 900 microns in length, dividing into two broad uteri which continue to posterior quarter of body where they give off oviducts and ovaries. Anus patent, subterminal, 15, 14 microns from caudal extremity.

MICROFILARIA (Geimsa, five specimens): Length 110-130 microns. Width 3-4 microns. Surrounded by delicate, hyaline sheath. Caudal end slightly attenuated, ending in rounded apex. Cephalic space absent. Positions of fixed points expressed as percentages of total length as follows: nerve ring 29-33%; excretory pore 42-46%; excretory cell 45-49%; inner body 66-74%; first rectal cell 75-82%; anus 86-90%.

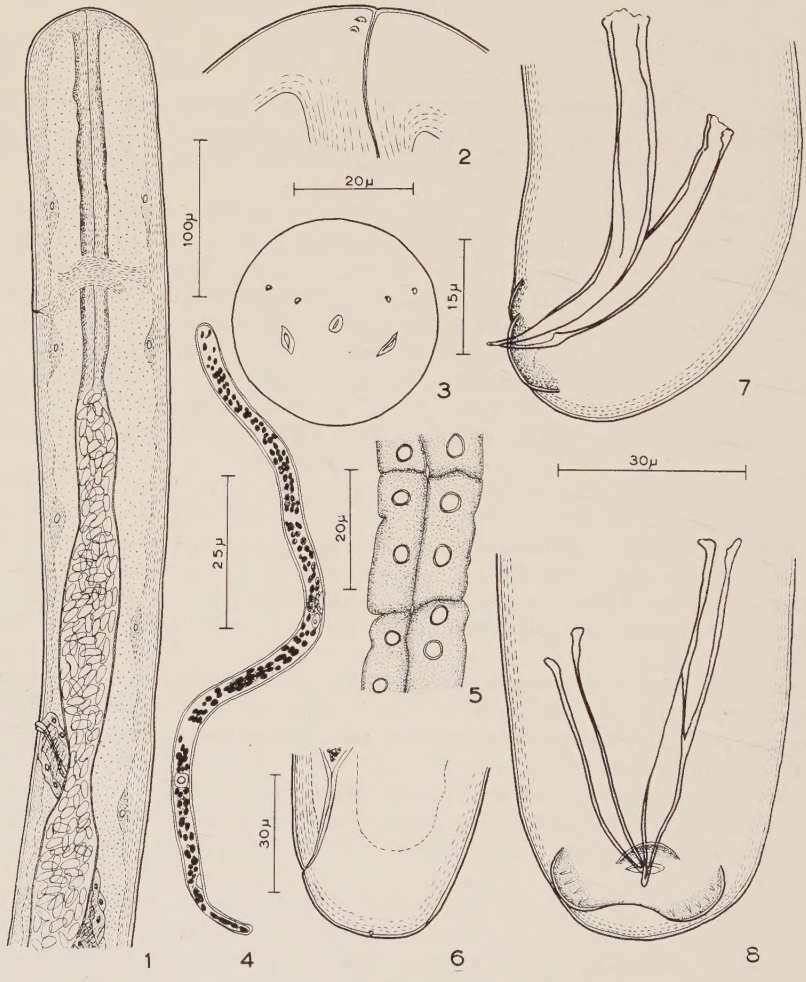
HOST: Java sparrow, *Padda oryzivora* (L). (Ploceidae).

LOCATION: Subcutaneous tissue in region of legs.

LOCALITY: Java; imported to St. Thomas, Ontario.

SPECIMENS: U.S.N.M. No. 39035.

COMMENTS: *Eufilaria mcintoshi* is readily distinguished from other described species. The spicules of *E. sergenti* Seurat, 1921 are much smaller in relation to the size of the caudal extremity, caudal swellings are absent in the male, and the vulva is in the oesophageal region. The distal half of the spicules of *E. micropennis* (Travassos, 1926) are broad rather than tapering as in *E. mcintoshi* and it is a much larger species. The anus of the female of *E. lari* Yamaguti, 1935 is terminal rather than subterminal, the oesophagus is considerably longer and the microfilaria shorter; the male is unknown. The caudal end of the male of *E. asiaticus* Singh, 1949 has two pairs of lateral swellings




*Eufilaria mcintoshi* n. sp.

Fig. 1. Anterior end female, lateral view (allotype); Fig. 2. Cephalic end female, right lateral view (paratype); Fig. 3. Cephalic end female, *en face* view (paratype); Fig. 4. Microfilaria from blood, Giemsa's stain; Fig. 5. Lateral field male, middle of body; Fig. 6. Caudal end female, lateral view (allotype); Fig. 7. Caudal end male, lateral view (holotype); Fig. 8. Caudal end male, ventral view (holotype).

as well as a terminal protuberance and the spicules are less subequal and double the size of those of *E. mcintoshi.* The anterior end of *delicata* Supperer, 1958 is bulbous and the microfilaria has a sharply pointed tail. *E. capsulata* (Annett, Dutton and Elliot, 1901) was not described in detail but the female was said to be 40 mm. in length, over twice the length of the female of *E. mcintoshi.* All species of *Eufilaria* are parasites of subcutaneous tissues of birds.

The microfilaria of *E. mcintoshi* failed to develop in *Aedes aegypti.*

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## Some Characteristics of the Early Phase of Migration of Larvae of *Ascaris suum**

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Among investigators who have studied the rate of migration and growth of larval *Ascaris suum* are Ransom and Foster (1920), Ransom and Cram (1921), Martin (1926), Roberts (1934), Sprent (1952) and Kelley *et al.* (1957). Hosts used by these authors included mice, guinea pigs, rabbits and pigs. Their studies, however, have not generally considered migration or growth of larvae during the first three days of infection. Our observations have indicated that three-day-old larvae show an unexpected pattern of migration and the present study was planned to describe in part this early migratory behaviour.

### MATERIALS AND METHODS

The *A. suum* eggs were obtained from uteri of adult female worms, embryonated on moist granular charcoal at 27°C. for a period of six weeks, then stored at 5°C. until used. The chitinous layer of the shell was removed by macerating the eggs for 16 hours at 37°C. in a solution consisting of equal parts "Clorox" (5.25% sodium hypochlorite) and 3% sodium hydroxide.

After the decoating process the eggs were washed through a sieve which separated them from the larger charcoal particles. The decoated eggs were then washed four times in tap water. Doses were prepared by dilution counts, and the eggs were administered to host animals by gavage.

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Published with the approval of the director as paper No. 981, Nebraska Agricultural Experiment Station.

Doses of 10,000 and 100,000 eggs were given to mice, rats, and eleven-day-old pigs. The pigs were obtained by hysterectomy, deprived of sows colostrum, and raised in strict isolation, using the method described by Young *et al.* (1955). This procedure prevented uncontrolled infection with *A. suum*.

All hosts were killed and examined 72 hours after infection. The number of larvae present in the liver and lungs of each pig and rat was estimated by trituration of the tissues and dilution counts, Kelley *et al.*, (1957). Contents of the stomachs of the rats were adjusted to five ml. and the number of larvae present estimated by counting those in a one-half ml. aliquot of the suspension. Homogenates of the entire liver and lungs of mice were prepared, and their volumes adjusted to 20 and 10 ml. respectively. The stomach contents were removed and their volumes adjusted to five ml. The number of larvae present in each organ was estimated by counting the larvae in a one-tenth aliquot of the material in each case.

Measurements were made of 25 randomly selected larvae from the liver and lungs of the pigs, and of 20 larvae from liver, lungs and stomach of the rats and mice. To obtain lengths of pooled larvae from mice, liver and lung homogenates and stomach contents were mixed in a single tube and twenty randomly selected larvae measured. In the case of piglets and rats, lengths of pooled larvae were calculated from the size and percentage present in each organ. Student's "t" test was used in all tests for significant differences.

## RESULTS

Data on the larvae from the three host types are summarized in Table 1.

Ingestion of the large egg dose resulted in accelerated migration in mice, but had no apparent effect in rats and piglets.

With the exception of one of the pigs and four of the mice which received the large egg dose, all animals contained larvae in the lungs which were significantly smaller, at the 1% probability level, than larvae in the liver. In hosts where larvae were found in the stomach (all of the high-dose mice and one of the high-dose rats), these larvae, with the exception of those in one of the mice, were significantly smaller, at the 1% probability level, than larvae in the lungs.

The large egg dose in mice caused a significant reduction in size of pooled larvae from liver, lungs, and stomach.

## DISCUSSION

The occurrence in the lungs of significantly smaller larvae than in the liver, and in the stomach of significantly smaller larvae than in the lungs is contrary to what one would normally expect. Ransom and Foster (1920) state that ". . . it is evident that there is a general increase in size with the lapse of time and with the progress of the larvae through the liver, lungs, trachea, and into the alimentary tract . . .". Some possible explanations for this size-difference phenomenon are: (1) a filtering action by the liver blood vessels, physically impeding the progress of the large larvae to a greater extent than the small; (2) the fact that the liver affords a more favorable growth environment for those larvae which by chance remain there longer; (3) a greater activity on the part of the small larvae, causing them to pass through the liver earlier than the large; or (4) the possibility that certain of the larvae, in moving into the intestinal wall, penetrate lymphatics rather than venules of the portal system, thus reaching the heart via the thoracic duct and by-passing the liver. Ransom and Cram (1921) noted the possibility of a migration through the

Table 1. Migration and growth of *Ascaris* larvae in baby pigs, rats, and mice three days after receiving 10,000 or 100,000 eggs.

| Host Type | No. of Animals | No. of Eggs Given | Total No. Larvae Recovered* | % Larvae in:* | | | Average Length of Larvae (in mm):* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Liver | Lungs | Stomach | Liver | Lungs | Stomach | Pooled |
| Pig | 3 | 10,000 | 8,038 (8,157) | 99.6 (0.12) | 0.4 (0.12) | 0 | 0.384 (0.010) | 0.298 (0.035) | -------- | 0.384 (0.010) |
| Pig | 3 | 100,000 | 24,376 (10,356) | 99.1 (0.35) | 0.9 (0.35) | 0 | 0.384 (0.054) | 0.318 (0.009) | -------- | 0.383 (0.052) |
| Rat | 3 | 10,000 | 919 (656) | 9.3 (11.6) | 90.7 (11.6) | 0 | 0.421 (0.064) | 0.253 (0.010) | -------- | 0.270 (0.033) |
| Rat | 4 | 100,000 | 3,708 (3,257) | 29.5 (44.6) | 70.3 (44.6) | 0.2 ---- | 0.389 (0.051) | 0.273 (0.022) | 0.235 -------- | 0.309 (0.083) |
| Mouse | 13 | 10,000 | 4,898 (1,671) | 81.9 (15.1) | 18.1 (15.1) | 0 | 0.388 (0.036) | 0.288 (0.014) | -------- | 0.359 (0.042) |
| Mouse | 13** | 100,000 | 30,736 (10,395) | 25.3 (16.6) | 74.4 (16.5) | 0.3 (0.2) | 0.293 (0.010) | 0.293 (0.025) | 0.245 (0.008) | 0.292*** (0.015) |

*Number given is the mean for all animals; the standard deviation is given in parentheses below each mean.
**Only seven survived and were examined three days after infection.
***Significantly smaller than pooled small-dose mouse larvae at 1% probability level.

lymphatics to the heart in commenting on the presence of larvae in mesenteric lymph nodes in 14 out of 18 infected guinea pigs.

An examination of the published data of other authors who have recorded measurements of larvae in the early period of migration furnishes some additional evidence of the more rapid migration of small larvae noted here. Roberts (1934) recorded, from the livers of guinea pigs and pigs, two-day-old larvae ranging from 0.27 to 0.31 mm long, while in the lungs the larvae measured from 0.27 to 0.305 mm. Three-day-old larvae in the same hosts ranged from 0.28 to 0.33 mm in the liver and from 0.245 to 0.335 in the lungs. Kelley *et al.* (1957) recovered larvae from the livers of two pigs two days after infection which averaged 0.33 mm long, while larvae from the lungs of those pigs averaged only 0.30 mm in length.

The acceleration of migration which accompanied large egg doses in mice but not in rats or pigs may have been caused by the large number of larvae entering the relatively small liver of the mouse. This massive migration may have, by sheer force of numbers, widened migration pathways in the liver which otherwise would have been effective in slowing the progress of the larvae.

The significantly reduced growth rate of pooled larvae which resulted from the large egg dose in mice may have been produced by the effects of crowding or by a lowering of the fitness of the environment provided for the larvae, caused by adverse physiological effects on the host of the massive larval migration.

## SUMMARY

Progress of migration and growth of larvae of *Ascaris suum* was determined in piglets, rats, and mice seventy-two hours after infection. Effects of egg doses of 10,000 and 100,000 were compared.

The 100,000 egg dose accelerated migration in mice, but not in rats or piglets.

In all three host types the 10,000 egg doses consistently produced larvae in the lungs which were significantly smaller than those in the liver. When larvae resulting from the 100,000 egg doses were found in the stomach, they were in nearly all cases significantly smaller than larvae in the lungs. Possible explanations for these size differences are discussed.

In mice, the 100,000 egg dose caused a significant reduction in size of pooled larvae from liver, lungs, and stomach.

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## A note on *Ogmocotyle ailuri* (Price, 1954) (Trematoda: Notocotylidae).

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In 1954 the writer described in abstract a species of notocotyloid trematode, which belonged to the genus *Ogmocotyle* Skrjabin and Shul'ts, 1933, from the lesser panda, *Ailurus fulgens*. Through a lapsus, apparently because of the similarity of the generic names *Ogmocotyle* and *Ogmogaster* (both notocotyloid), the name *Ogmogaster ailuri* was proposed for the parasite.

The host, *Ailurus fulgens*, was a young adult which died in the National Zoological Park, Washington, D. C. on May 21, 1957, and was listed in the records of the U. S. National Museum as Accession No. 252091. The specimen had been obtained from a New York dealer in wild animals but beyond this no information was available as to its original source.

In view of the fact that the lesser panda was a relatively rare animal in zoological gardens and because the Museum authorities wished to utilize the carcass for anatomical studies, a complete necropsy was not possible. However, on May 27, 1927 (erroneously given in the abstract as April 27) the writer was permitted to flush out the intestinal tract, by means of a tube inserted into the duodenum, and a single specimen of the trematode referred to above was recovered in the washings.

The purpose of this note is to present a more complete description of the species than was possible in the abstract.

*Ogmocotyle ailuri* (Price, 1954) Fig. 1.

SYNONYM. *Ogmogaster ailuri* Price, 1954

DESCRIPTION. Body scoop- or boat-shaped, 1.3 mm long by 0.73 mm wide. Cuticle smooth and without spines. Oral sucker subterminal, 0.112 mm in diameter; esophagus slender, 0.13 mm long; intestinal branches slender but could not be followed beyond level of anterior transverse uterine loop. Genital aperture to left of median line, about 0.64 mm from anterior end of body. Cirrus pouch somewhat crescent-shaped, about 0.6 mm long by 0.15 mm wide, the distal portion almost completely filled by the seminal vesicle and the transverse portion almost entirely occupied by the pars prostatica; cirrus unarmed. Testes 0.29 mm long by 0.096 mm wide, situated as in other notocotyloids. Ovary deeply lobed, about 0.1 mm long by 0.24 mm wide, situated between posterior ends of testes. Mehlis' gland about one-third as large as orary and antero-dorsal to it. Vitellaria consisting of relatively large follicles, forming band across body dorsal and largely anterior to testes. Uterus consisting of more or less regular transverse loops extending laterally to near margin of body, almost filling zone between anterior margins of testes and cirrus pouch. Metraterm prominent, transverse, about 0.24 mm long by 0.08 mm wide. Eggs 0.018 mm long by 0.011 mm wide, many with long filament at anterior and posterior poles and others with a long filament at antopercular pole and 2 shorter filaments at opercular pole.

HOST. Lesser panda, *Ailurus fulgens*.

LOCATION. Intestine.

LOCALITY. National Zoological Park, Washington, D. C.

HOLOTYPE. U. S. National Museum Helminthological Collection No. 27777.

DISCUSSION. The genus *Ogmocotyle* was proposed by Skrjabin and Shul'ts

(1933) for *O. pygargi* which had been collected from the small intestine of *Capreola pygargus bedfordi* in Siberia. In the same year Yamaguti (1933) proposed the genus *Cymbaforma* for a notocotyloid trematode, *C. sikae,* which had been obtained from the upper portion of the small intestine of *Sika nippon* in Japan. Ruiz (1946) regarded the two species as congeneric and pointed out that *Ogmocotyle* Skrjabin and Shul'ts had priority of a few months over *Cymbaforma* Yamaguti. Skrjabin (1953) regarded Yamaguti's species as a synonym of *O. pygargi,* an action that appears correct since there are insufficient differences to warrant regarding the two species as distinct.

Bhalerao (1942) described as *Cymbaforma indica* a species from the small

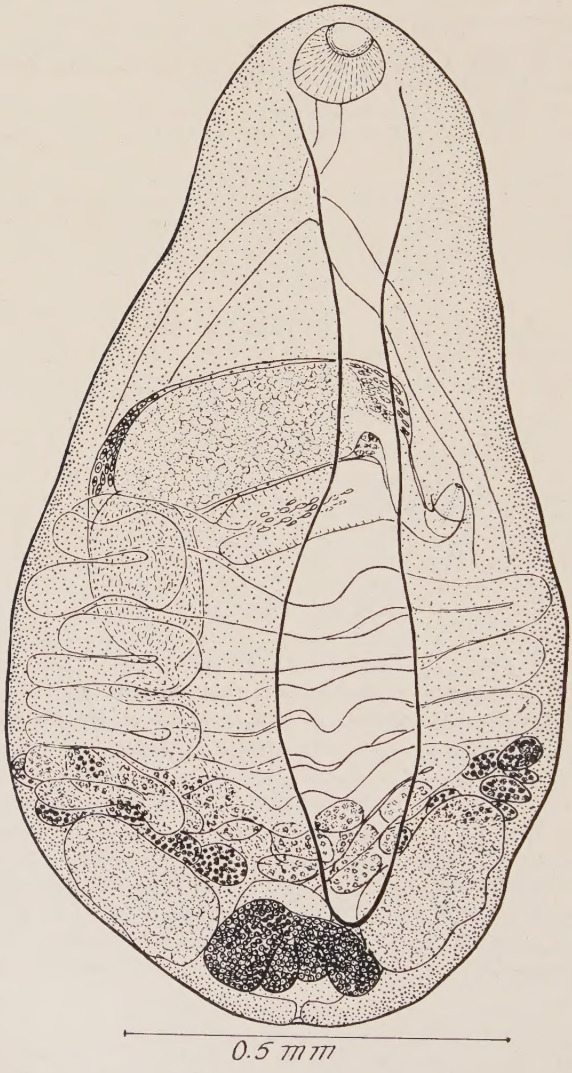


Fig. 1. *Ogmocotyle ailuri.* Complete worm, ventral view. Original.

intestine and bile ducts of sheep, goats and cattle in India. This species was subsequentlyplaced in the genus *Ogmocotyle* by Ruiz (loc. cit.). Concerning the frequency of occurence of this parasite, Balerao (1948) stated that "*Cymbiforma indica* ..... is very common in goats and sheep and rare in cattle at Mukteswar."

*Ogmocotyle ailuri* resembles *O. indica* in many respects and may eventually be shown to be identical in spite of the wide difference in hosts. The principal character which seems to differentiate the two forms is the cirrus pouch, this structure being situated more transversely in *O. ailuri* than in *O. indica.*

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## Acetylcholinesterase in Plant-Parasitic Nematodes and an Anticholinesterase from Asparagus*

R. A. ROHDE**

The enzyme acetylcholinesterase and a cholinergic system of nerve impulse transport appear to be almost universally distributed throughout the animal kingdom. At least three species of animal-parasitic nematodes have been found to have acetylcholine in somatic and nerve tissue (Mellanby, 1955). Mellanby showed that in *Ascaris lumbricoides* the highest concentration of acetylcholine occurred in the region of the nerve ring and sensory terminals. Krotov (1957) found that intact individuals of *Ascaris* sp. are sensitive to added acetylcholine and that this effect is enhanced by pretreatment with proserine. He was able to demonstrate an acetylcholine splitting enzyme which was highly concentrated in the nerve ring and sensory terminals of the lips. While the above experiments do not meet all of the criteria of Bacq (1947) for the identification of a cholinergic system, there is very strong evidence for this system, at least in *Ascaris.*

No information is available concerning the presence of acetylcholine or acetylcholinesterase in plant-parasitic nematodes. Organic phosphate insecticides which inhibit cholinesterase in insects and other animals are highly toxic to soil nematodes (Christie, 1959). A possible assumption is that the mode of action of these chemicals is similar in all groups of animals. It is the purpose of this paper to present evidence that an acetylcholine-splitting enzyme, sensitive to cholinesterase inhibitors, is present in several species of plant-parasitic nematodes.

*Scientific Article No. *A805*, Contribution No. *3076* of the Maryland Agricultural Experiment Station, Department of Botany.

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## MATERIALS AND METHODS

Acetylcholinesterase was determined histochemically by the use of Gomori's modification of Koelle and Friedenwald's substrate (Gomori, 1952). Intact nematodes were placed in this substrate, a buffered solution of copper ion and acetylthiocholine, for 24 hours. Enzymatic hydrolysis of acetylthiocholine yields thiocholine in the form of copper mercaptide. After rinsing in saturated ammonium sulfate, specimens were transferred to a solution of concentrated ammonium sulfide for 15 minutes. Dark brown cupric sulfide was deposited in sites where thiocholine was released while areas which did not react with the substrate were unstained. Time intervals in solution were varied, those reported above being most satisfactory.

Three different compounds were studied in regard to their effect on the staining reaction described above. Thimet® phorate (0,0-diethyl S-(ethylthio) methyl phosphorodithioate) was selected as a known cholinesterase inhibitor,

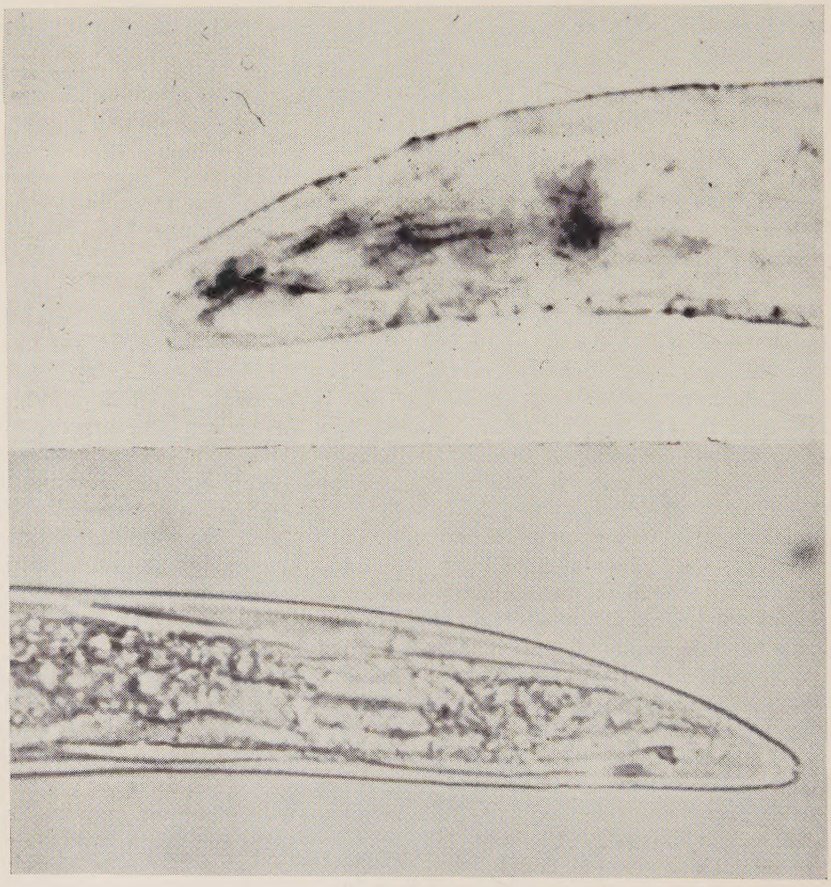

Figure 1. Nematode specimens stained to show location of acetylcholinesterase. Upper: Anterior end of *Trichodorus christiei* showing region of nerve ring stained darkly. Lower: Posterior end of *Pratylenchus penertans* showing darkly stained phasmid and underlying pouch.

and formalin because it denatures most proteins but has little effect on acetylcholinesterases (Colowick and Kaplan, 1957). The third compound used was a glycoside extracted from soil around the roots of the asparagus variety Mary Washington. This compound is toxic to nematodes but death is preceded by abnormal twitching and paralysis, indicating a possible effect on the nervous system (Rohde and Jenkins, 1958). The material is also an inhibitor of human plasma cholinesterase.†

To study the effects of these three compounds, specimens were divided into 4 groups and placed for one hour in distilled water, 3 per cent formaldehyde, 0.5 per cent commercial thimet, and 0.1 per cent asparagus toxin. After these pretreatments, the nematodes were placed in the acetylthiocholine substrate.

## Results and Discussion

Positive reactions, indicating the presence of cholinesterase, were obtained with all species of nematodes tested, including *Trichodorus christiei, Pratylenchus penetrans, Xiphinema americanum, Dorylaimus* sp., and *Helicotylenchus nannus.* The most intensively stained areas were the various parts of the nervous system, including the nerve ring and associated ganglia, amphids, phasmids and papillae on the lips and body surface. (Fig. 1).

Pretreatment with 3 per cent formaldehyde in many cases intensified the staining reaction when compared with the distilled water controls. No reaction occurred after pretreatment with thimet or asparagus extract, an indication that the enzyme had been inactivated.

The presence of an enzyme capable of hydrolyzing acetylthiocholine (and presumably therefore acetylcholine) which is inactivated by cholinesterase inhibitors and is concentrated in nerve tissue is not complete evidence of an active cholinergic system, but indicates a strong possibility that this is the case. Further work is necessary to determine the specificity of the enzyme or enzymes and its behavior in the presence of specific inhibitors.

Mary Washington asparagus is resistant to attack by plant parasitic nematodes because of the release of the toxic glycoside, used in the above experiments, into the root zone (Rohde and Jenkins, 1958). The above results support the theory that the mechanism of toxicity is an interference with acetylcholinesterase.

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†Anticholinesterase activity was measured by an unpublished method of Dr. A. N. Curry, American Cyanamid Company, Stamford, Connecticut.

# A New Stylet-Cercaria, *C. edgbastonensis* from *Lymnaea stagnalis* (L.)

P. NASIR*

In a study of larval trematodes of *Lymnaea stagnalis* (L) from Edgbaston Pool (a small lake near the University of Birmingham, England) three species of stylet-cercariae were encountered. Two of these, *Cercaria pseudarmata* (Brown, 1926) and the cercaria of *Plagiorchis* (*M*) *megalorchis* (Rees, 1952) have been previously described and will not be dealt with in this paper. The third cercaria is described and is here proposed as a new species: *Cercaria egbastonensis*.

## MATERIAL AND METHODS

Snails, *Lymnaea stagnalis*, were isolated in water in 2″ by 1″ glass tubes and checked for cercarial emergence.

All cercariae were studied alive unstained or with the aid of the vital stains, methylene blue, nile blue sulphate and neutral red. Neutral red 0.05% was very helpful for identification of penetration glands and their ducts.

*Cercaria edgbastonensis*, n. sp. (fig. 1 to 2)

Measurements in mm. are based on twenty living specimens naturally emerged from their host.

DIAGNOSIS: Stylet-cercaria of Conniae subdivision of Polyadenous cercariae. Body, 0.096 to 0.296 by 0.064 to 0.104. Tail, without a finfold, 0.064 to 0.264 by 0.024, lodged in a subterminal caudal depression; the latter 0.017 to 0.026 by 0.015 to 0.028, flanked by caudal pockets, lining of which produced into fine needle-like processes. Cuticle of body, with transverse rows of spines, most prominent in preace-tabular region; fifteen transverse rows of "flagellets," 0.057 to 0.088 long, all over body. Stylet-organ, javelin-shaped, without a basal bulb, 0.025 to 0.027 long; width of shaft, 0.004, width of shoulder, 0.006. Oral sucker, 0.035 to 0.043 in diameter, ventral sucker 0.027 to 0.033; the latter lying behind the middle of the body. Prepharynx, very short. Pharynx, 0.012 to 0.016 in diameter. Esophagus, 0.012 to 0.025 in length, bifurcating midway between suckers; intestinal ceca, short stump-like appendages, never extending to ventral sucker. Penetration gland cells, eight pairs, with granular contents and vesicular nuclei, mostly anterior to ventral sucker. Cystogenous gland cells, granular, uninucleated, occupying dorsal and lateral margins of body, especially abundant in postace-tabular region. Large globular concretion bodies present. Genital primordia, two masses of cells, one anterior and one posterior to ventral sucker, connected by strand of cells running slightly dorsal and laterally around ventral sucker. Excretory bladder with posterior rectangular portion, joined by narrow median portion to two anterior lateral horns that reach posterolateral margins of ventral sucker. Posterior rectangular portion opens into excretory pore through a narrow posterior tube. Excretory pore located at anterior dorsal border of caudal depression. Main lateral excretory tubes arise from middle of anterior ends of corresponding horns and divide into anterior and posterior lateral collecting

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This report is taken from a thesis submitted to the University of Birmingham, England, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
The author expresses his appreciation to Dr. J. Llewellyn, Zoology Department, University of Birmingham, England, for the help given during the course of this work and to Prof. D. L. DeGiusti. Wayne State University, for help in preparing the manuscript.
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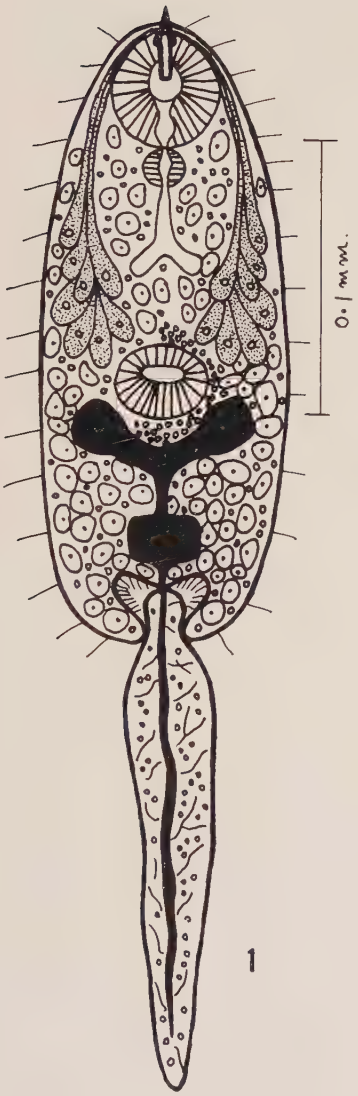


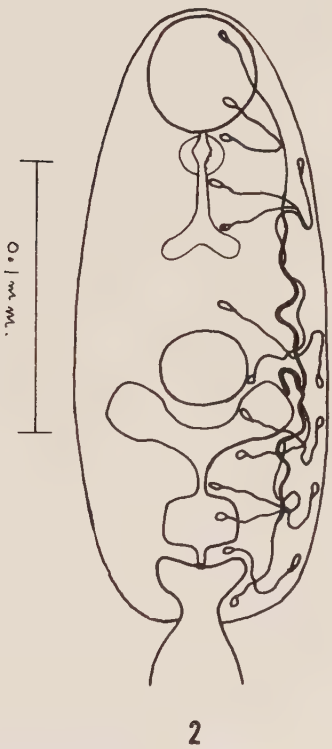

Figure 1. General anatomy of *Cercaria edgbastonensis* n. sp.
Figure 1a. Stylet, dorsal view.
Figure 1b. Stylet, side view.
Figure 2. Excretory system of cercaria, shown on one side only.
(All figures drawn with aid of *camera lucida* except the flame cell pattern in figure 2, drawn free hand.)

tubules at equator of ventral sucker. Flame cells, thirty-six, represented by formula 2 (3-3-3)-(3-3-3) = 36. Caudal excretory duct absent. Development, in sausage-shaped sporocysts, 0.027 to 0.08 long. Host, *L. stagnalis* (*L*). Encystment, in *L. stagnalis, Chironomus* larvae as well as free in water; cysts measure 0.096 (0.104-0.112) in diameter.

Large numbers of immature cercariae were common free in the liver spaces of snails which harbored this particular infection. The body of these immature cercariae was more transparent than that of naturally emerged cercariae, a fact also observed by Stunkard (1930) in the cercariae of *Cryptocotyle lingua*. This is probably due to the fact that the glands of the body had not yet been filled with the secretory material. Thus it appears that a period of extra-sporocystic existence may be necessary for the completion of the development of *Cercaria edgbastonensis*.

The cercariae emerge, in great number during the evening, at night and early morning hours. However, on occasion a small number of cercariae may be observed emerging during the day.

Species: Within the Polyadenous group, related to *Cercaria edgbastonensis* are: *Cercaria isocotylea* (Cort, 1914), *C. micropharynx* (Faust, 1917), *C. indicae XVII* (Sewell, 1922), *C. helvetica* XVII (Dubois, 1929), *C. acanthocoela* (Miller, 1935), *C. concavocorpa* (Sizemore, 1936), *C. plagiorchis muris* (Tanabe) and *C. plagiorchis micracanthos* (Macy) as described by McMullen (1937), *C. brevicauda* (Byrd and Reiber, 1940), *C. nolfi* (Brooks, 1943), *C. conniae* (Brooks, 1943), *C. goodmani* (Najarian, 1952), cercaria of *Plagiorchis* (*M*) *megalorchis* (Rees, 1952), the cercaria of *Plagiorchis parorchis* (Macy, 1956), the cercaria of *Opithioglyphe locellus* as described by Macy and Moore (1958), and the cercaria of *Plagiorchis vespertilionis parorchis* (Macy, 1960). Of these sixteen cercariae *C. edgbastonensis* shows closest resemblance, on the basis of the size and shape of the stylet, with *C. goodmani*, and the cercaria of *P.* (*M.*) *megalorchis*, a detailed comparison of these cercariae is given below.

*Cercaria edgbastonensis* is smaller than the cercaria of *Plagiorchis* (*M*) *megalorchis*. It further differs in having a smaller stylet than the cercaria of *P.* (*M.*) *megalorchis*. The intestinal ceca of *C. edgbastonensis* are very short, while in *P.* (*M.*) *megalorchis* they extend to the posterior end of the body.

The shape and size of the stylet in *Cercaria goodmani* is identical with that of *C. edgbastonensis*—these two cercariae are indistinguishable on the basis of this characteristic alone. However, certain differences afford sufficient grounds for separation of these two species. *C. goodmani* is only about half the size of *C. edgbastonensis*. The oral and the ventral suckers in *C. goodmani* are larger than those of *C. edgbastonensis*. The main excretory tubes in *C. goodmani* arise subterminally from the horns of the excretory vesicle, whereas in *C. edgbastonensis* the main lateral tubes arise terminally from the horns of the excretory vesicle. The two cercariae differ biologically. *C. goodmani* has not been observed to encyst in open water whereas *C. edgbastonensis* does encyst in open water as well as in snails and *Chironomus* larvae.

In conclusion, it can be stated that morphologically, *C. edgbastonensis* more closely resembles *C. goodmani* found in *Lymnea palustris* in Michigan than the cercaria of *P.* (*M.*) *megalorchis* found in *L. pereger* in Wales.

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# Longevity *in vitro of Ditylenchus dipsaci* (Kühn) Filipjev from Narcissus

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Previous studies (Hasting, 1942) of survival of the pre-adult stage of *Ditylenchus dipsaci* in the form of nematode "wool" as found in the basal region of narcissus bulbs have shown 100% mortality after storage in glass vials at room temperature for a period of four years. Fielding (1951) records survival of *D. dipsaci* in dried plant tissues for a period of 23 years, indicating that maintenance of life in these nematodes is affected by obscure factors of environment. Further studies (Bosher and McKeen, 1954) showed that *D. dipsaci* in the dry state from narcissus "wool" and in certain media survived freezing to -80° C. followed by vacuum dehydration (lyophilization) and storage *in vacuo* in sealed tubes for a period of up to 28 days. It was postulated that lyophilization may prove to be of value as a method for the maintenance of stock cultures for laboratory investigations. Survival of these nematodes in relation to environment has been further investigated as follows.

## MATERIALS AND METHODS

Clusters of nematode "wool" from narcissus collected in 1951 were divided into two portions and placed in separate screw-cap glass vials. One portion was placed on a laboratory shelf at room temperature and one lot was stored in a household type refrigerator at 2°-4° C. Portions of the clusters were removed at 2-year intervals until 1958 and the percentage of motile nematodes recorded after 48 hours immersion in shallow tap water as shown in Table 1.

Sealed tubes of nematodes *in vacuo* prepared in 1953 by lyophilization at -80° C., held in storage at room temperature for 5 years, were opened and viability of the nematodes was determined by immersion in shallow tap water. Table 2 shows the percentage of nematodes that regained motility as compared with similar samples examined in 1953 shortly after lyophilization.

Table 1. Revival of *Ditylenchus dipsaci* from narcissus "wool" *in vitro* in relation to time and storage temperature.

| Storage | % revival after storage for: 1 yr. | 3 yrs. | 5 yrs. | 7 yrs. |
|---|---|---|---|---|
| Room temperature, approx. 21° C. | 86 | 38 | 3 | 0 |
| Refrigerator at 2°-4° C. | 89 | 86 | 81 | 78 |

## RESULTS

Nematodes that revived from the material stored at low temperature regained active motility within 24 hours after being placed in water. The small percentage that revived after five years at room temperature exhibited comparatively feeble movement between 24 and 48 hours after immersion.

Nematodes from tubes of the lyophilized series were poured into pots containing bulbs of narcissus var. King Alfred from nematode-free stock. Examination of the bulbs after one year's growth showed populations of *D. dipsaci* of all stages from eggs to adults in bulbs inoculated with nematodes from lyophilized dry wool and dry wool in beef serum. No nematodes were found in bulbs inoculated with *D. dipsaci* treated as dry wool in sucrose or in water.

Table 2. Revival of *Ditylenchus dipsaci* from narcissus "wool" subsequent to lyophilization at -80° C. in relation to time of storage *in vacuo* at room temperature.

| Nematode state | % revival after storage for: 28 days | 5 years |
|---|---|---|
| Dry wool | 80 | 20.4 |
| Dry wool in beef serum | 80 | 11.8 |
| Dry wool in 50% sucrose | 90# | 0 |
| Dry wool in water | 30 | 0 |

#12 days storage.

## DISCUSSION

The results presented herein are a further indication of the remarkable resistance to unfavorable environment of the pre-adult stage of *D. dipsaci* in the dry state.

Storage at low temperature is indicated as a more effective method for maintenance of visability of these nematodes than lyophilization at extreme cold followed by storage *in vacuo*.

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## Isolation of *Trichomonas gallinae* from the White-winged Dove, *Zenaida a. asiatica*

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On September 13, 1959, a series of 17 throat swabbings were obtained from white-winged doves shot by hunters near Edinburg, Texas. The swabs were placed in tubes of Diamond's trichomonad medium (Jour. Parasit., 43: 488-490, 1957) and mailed to the Patuxent Research Refuge for examination. Upon arrival at the refuge, two and three days later, the tubes were placed in an incubator at 37.5° C. The following day the cultures were examined for trichomonads.

Six of the 17 were positive for *Trichomonas gallinae;* all six were swabbings from the 10 adult doves that on external examination were normal and fat. No *Trichomonas gallinae* was isolated from the 7 immature birds.

Although *T. gallinae* frequently has been isolated from mourning doves, this is, to our knowledge, the first report of its isolation from the white-winged dove.

Thanks are extended to Mr. David Blankinship, Texas Game and Fish Commission, for his aid in collecting several of the samples.

## Some Trematodes from Otters in Southern Rhodesia including a new Strigeid, *Prudhoella rhodesiensis*, n. gen., n. sp.

MARY BEVERLEY-BURTON (Mrs. D. F. Mettrick)*

Two otters were examined in March and April, 1959. The otters were trapped at the Henderson Fisheries Research Station, Mazoe, Southern Rhodesia and large numbers of trematodes were recovered from the intestines. The worms were fixed in cold formal-acetic, under slight coverslip pressure and stained with Kirkpatrick's carmalum. Facial and sagittal sections were cut of several specimens using the Polyethylene glycol distearate embedding technique (Steedman, 1957). All measurements, unless otherwise stated, are in millimeters.

*Prudhoella*, n. gen.

DIAGNOSIS: Diplostomatidae Poirier, 1886; body cylindrical; forebody not clearly demarcated from hindbody. Forebody cup shaped with a shallow median ventral cleft; "holdfast" organ tongue-like and protrusible. Hindbody longer than forebody. Oral sucker small, situated on anterior margin. Pharynx present. Ventral sucker poorly developed. Ovary and Mehlis' gland anterior to testes which lie one behind the other. Ascending limb of uterus extends to posterior margin of forebody; descending limb ventral to testes. Seminal vesicle opens into uterus, via a thin walled ejaculatory duct, to form a short hermaphrodite duct. There is no genital bulb or genital cone. Vitelline follicles occur in posterior half of forebody, "holdfast" and anterior region of hindbody. The name *Prudhoella* is proposed in honour of Mr. S. Prudhoe of the British Museum (Natural History).

*Prudhoella rhodesiensis*, n. sp.

SPECIFIC DESCRIPTION: With characters of the genus. Body 3.12-4.27 long; forebody 1.10-1.72 long by 0.56-0.57; hindbody 2.02-2.50 by 0.52-0.69 (figs. 1 and 2). Forebody cup shaped, often embedded in intestinal wall of host. "Holdfast" tongue-like; 0.84-1.11 long by 0.41-0.49; can be protruded beyond anterior margin of forebody or completely withdrawn. Oral sucker weakly muscular, 0.05 long by 0.09-0.10; pharynx conspicuous, 0.12-0.13 by 0.12-0.13 diameter; oesophagus 0.02-0.04 long; intestinal caeca long, extending to level of seminal vesicle. Ventral sucker small, 0.03-0.04 by 0.04, situated 0.07-0.08 behind the intestinal bifurcation. Testes wider than long, one behind the other in posterior half of hindbody. Testes lobed with median ventral groove to accommodate descending uterus and vas deferens. Anterior testis 0.31-0.41 by 0.43-0.63; posterior 0.35-0.48 by 0.43-0.65. Vas deferens, 0.04-0.09 in diameter, forms a transverse loop anterior to ovary before passing posteriad to the seminal vesicle. Seminal vesicle 0.12-0.19 in diameter forms a transverse loop posterior to testes. From the seminal vesicle the ejaculatory duct, 10 microns in diameter, joins with the uterus to form a short hermaphrodite duct, 0.04-0.05 long, which opens medianly in ventral wall of bursa copulatrix. There is no genital cone or genital bulb (fig. 3). Opening of bursa copulatrix

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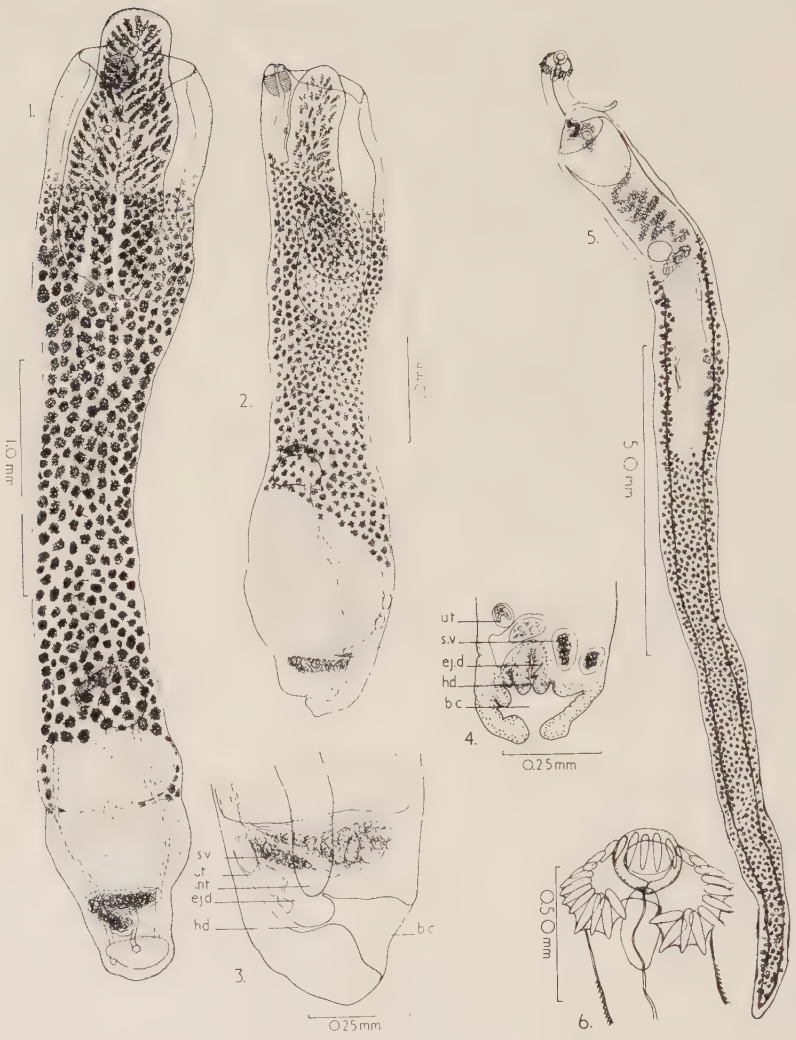
The present work was carried out during the tenure of a Research Fellowship sponsored by the Nuffield Foundation. The author is indebted to Mr. S. Prudhoe of the British Museum (Natural History) and Dr. A. Maar, Henderson Fisheries Research Station, Mazoe, Southern Rhodesia.




*Prudhoella rhodesiensis*, n. gen., n. sp.

Fig. 1. Entire. Dorsal view; Fig. 2. Entire. Lateral view; Fig. 3. Lateral view of bursa copulatrix.

*Cynodiplostomum namrui* Kuntz and Chandler, 1956.

Fig. 4. Facial section showing genital cone and bursa copulartrix.

*Baschkirovitrema incrassatum* (Diesing, 1850) Skrjabin, 1944.

Fig. 5. Entire. Ventral view; Fig. 6. Head collar. Ventral view.

b.c. bursa copulatrix; ej.d. ejaculatory duct; h.d. hermaphrodite duct; i.c. intestinal caecum; s.v. seminal vesicle; ut. uterus.

a transverse, dorsal slit up to 0.21 in diameter. Ovary smooth, 0.12-0.22 by 0.17-0.27, situated dorsally, immediately anterior to testes, on right or left of body. Oviduct coiled; Laurer's canal opens dorsally on right or left of mid-line. Mehlis' gland diffuse, lateral to ovary. Ascending limb of uterus, coiled initially, extending to posterior region of forebody; descending uterus passes, ventral to testes, to the hermaphrodite duct. Vitelline follicles dorsal, ventral and lateral, extending from the posterior half of forebody, throughout the "holdfast" to the testicular region of the hindbody. Dorsally vitellaria end just in front of anterior testis; ventrally the follicles extend to the intertesticular level. Transverse vitelline ducts present between testes; longitudinal duct, ventral to anterior testis, maybe distended in the ovarian region to form a vitelline reservoir. Eggs pale brown, 98-108 microns by 56-69 microns.

Hosts: *Lutra* (*Hydrictis*) *maculicollis maculicollis* Lichtenstein and *Aonyx capensis capensis* (Schinz).

Location: Small intestine.

Locality: Henderson Fisheries Research Station, Mazoe, Southern Rhodesia.

Co-types: To be deposited in the collection of the British Museum (Natural History).

Number Recovered: 286 specimens from *Lutra maculicollis* and 4 from *Aonyx capensis*.

Discussion: This new genus shows affinities with the genera *Alaria* Schrank, 1788 and *Enhydridiplostomum* Dubois, 1944, which occur in mustelids of the New World. *Prudhoella* has no pseudosuckers and is thus separate from both the above genera.

Chandler (1942) and Chandler and Rausch (1946) suggest that there is no sound basis for the division of the family Diplostomatidae Poirier, 1886 into the subfamilies Diplostomatinae Monticelli, 1888, and Alariinae Hall and Wigdor, 1918. Dubois (1938, 1953) recognises both these subfamilies.

The key given by Dubois (1953) for the separation of the Alariinae from the Diplostomatinae is as follows:—

"Parasites d'Oiseaux. Foll. vitgl. répartis dans les deux segm. du corps ou confinés dans le segm. post. Org. trib. petit à moyen, s'ouvrant générmt. par une fente médiane ..... Diplostomatinae

Parasites de Mammifères. Foll. vitgl. confinés ou tendant à se confiner dans le segm. ant. Org. trib. se développant jusqu' à l'hypertrophie et le massiveté, avec occlusion (la fente se réduisant souvt. à un sillon médian) ..... Alariinae"

The present genus, on the basis of the distribution of the vitellaria, should, therefore, belong to the subfamily Diplostomatinae but, as it occurs in mammals it can equally well be placed in the Alariinae. Chandler and Rausch (1946) point out similar anomalies in the genera *Fibricola* Dubois, 1932 and *Alaria* Schrank, 1788 to demonstrate the artificiality of using the distribution of the vitellaria and adaptation to mammals as criteria for the separation of the subfamily Alariinae.

It is suggested that the concept of the subfamilies Diplostomatinae and Alariinae, as recognised by Dubois (1938, 1953), is not tenable, and, in agreement with Chandler and Rausch (1946), the subfamily Alariinae should be supressed.

*Cynodiplostomum namrui* Kuntz and Chandler, 1956.

Description: Body 1.05-1.59 long by 0.46-0.66. Forebody 0.63-0.92 long, not clearly demarcated from hindbody, 0.41-0.67 long. Oral sucker 0.08-0.09

long by 0.08-0.11; pharynx 0.07-0.08 by 0.06-0.07; oesophagus absent; intestinal caeca extend to level of bursa copulatrix. Pseudo-suckers 0.06-0.09 in diameter. Ventral sucker 0.07-0.09 by 0.10-0.12, situated 0.25-0.46 from anterior margin of body. "Holdfast" oval 0.25-0.28 by 0.17-0.19. Anterior testis oval, 0.17-0.19 long by 0.15-0.22, situated in anterior part of hindbody on right or left side, partly dorsal to posterior testis. Posterior testis bilobed; each lobe 0.19-0.26 long by 0.12-0.20; lobes connected by narrow, dorsal isthmus. Seminal vesicle voluminous, coiled, up to 0.09 in diameter, dorsal to posterior testis. Ejaculatory duct joins with uterus to form hermaphrodite duct which crosses the genital cone (fig. 4). Ovary oval, 0.07-0.10 by 0.11-0.12, situated in anterior region of hindbody. Mehlis' gland dorsal, posterior to ovary. Laurer's canal opens dorsally at level of Mehlis' gland. Uterus runs forward between ovary and anterior testis to anterior margin of hindbody, then posteriad to genital cone. Genital cone with thick, muscular walls protrudes into lumen of bursa copulatrix from ventral side. Bursa copulatrix opens to exterior by subterminal pore. Vitelline follicles confined to posterior region of forebody. Transverse vitelline ducts meet medianly to form a vitelline reservoir situated just inside the hindbody. Eggs few, up to 17 in number, 102-116 microns by 53-67 microns.

Host: *Lutra* (*Hydrictis*) *maculicollis maculicollis* Lichtenstein.

Location: Small intestine.

Locality: Henderson Fisheries Research Station, Mazoe, Southern Rhodesia.

Number Recovered: 24 specimens from a single host.

Discussion: *Cynodiplostomum namrui* was described by Kuntz and Chandler (1956) from cats and dogs in Egypt. The present material is slightly larger than the type material so that a brief re-description has been included. *C. namrui* has not previously been recorded from a mustelid and is a new record for Southern Rhodesia.

*Baschkirovitrema incrassatum* (Diesing, 1850) Skrjabin, 1944.

Description: Body elongate, slender, 12.50-16.55 long by 0.96-1.23 (fig. 5). Cuticle spinous anteriorly; spines up to 21 microns long. Head collar reniform, 0.51-0.56 diameter with 27 spines (fig. 6). Corner spines; 2 ventral groups with 4 spines in each, of these 2 are oral in position and 2 aboral; lateral aboral spines 123-154 microns long by 35-37 microns, other corner spines 108-140 microns by 28-32 microns. Lateral marginal spines; 2 groups with 6 spines in each arranged in a single row; 105-130 microns by 28-32 microns. In any one specimen there is a progressive increase in size from lateral spine 1 to lateral spine 6. Dorsal spines 7, arranged in a double row; 4 oral and 3 aboral; median spine aboral. Oral spines 116-144 microns long, aboral spines 109-137 microns by 30-34 microns. Oral sucker 0.19-0.22 by 0.22-0.26; prepharynx variable, up to 0.12 long; pharynx 0.22-0.29 by 0.12-0.21; oesophagus up to 0.87 long; intestinal caeca extend nearly to posterior margin of body. Ventral sucker deep, muscular and conspicuous; situated in anterior fifth of body; 0.87-1.17 by 0.81-0.95 diameter. Testes elongate, smooth or irregular in outline; situated, one behind the other, in anterior half of body. Anterior testis 1.12-1.50 by 0.42-0.48; posterior testis 1.15-1.40 by 0.32-0.42. The two vasa deferentia arise from the lateral borders of the testes and unite just posterior to the cirrus sac. Cirrus sac variable in shape and

Table I. A comparison of the measurements of *Baschkirovitrema incrassatum* as given by Braun, 1901 and the present material.

| | Braun (1901). | Present Material. |
|---|---|---|
| Body length | 7-19 | 12.50-16.55 |
| Number of head spines | 27 | 27 |
| Corner spines | 104 X 31$\mu$ | 108-154 X 28-37$\mu$ |
| Lateral spines | 52 X 15$\mu$ (smallest) | 105-130 X 28-32$\mu$ |
| Dorsal spines | 83-93 X 21$\mu$ | 109-144 X 30-34$\mu$ |
| Oral sucker: length | 0.166-0.25 | 0.19-0.22 |
| Oral sucker: diameter | 0.187-0.208 | 0.22-0.26 |
| Ventral sucker: length | | 0.87-1.17 |
| Ventral sucker: diameter | 1.0 | 0.81-0.95 |
| Pharynx: diameter | 0.073-0.083 | 0.12-0.21 |
| Testes: length | 1.0 | 1.12-1.50 |
| Testes: diameter | 0.4 | 0.32-0.48 |
| Ovary: length | 0.33 | 0.32-0.39 |
| Ovary: diameter | 0.266 | 0.32-0.37 |
| Eggs: | 104 X 73$\mu$ | 108-123 X 54-62$\mu$ |

size, 0.48-1.43 by 0.24-0.29; filled by internal vesicula seminalis. Cirrus unarmed, up to 2.85 long. Ovary rounded, 0.32-0.39 by 0.32-0.37. Mehlis' gland diffuse, posterior to ovary. Uterus with short descending coil; ascending uterus forms transverse intercaecal slings between ovary and ventral sucker. Initial coils of uterus function as a receptaculum seminis. Metraterm opens at common genital pore situated just anterior to ventral sucker. Vitelline follicles extend from ovarian region to posterior margin of body, filling all available space behind testes. Transverse vitelline ducts unite behind ovary to form a small triangular reservoir. Eggs 108-123 microns by 54-62 microns.

HOSTS: *Aonyx capensis capensis* (Schinz) and *Lutra* (*Hydrictis*) *maculicollis maculicollis* Lichtenstein.

LOCATION: Small intestine.

LOCALITY: Henderson Fisheries Research Station, Mazoe, Southern Rhodesia.

NUMBER RECOVERED: 92 specimens from *Aonyx capensis* and 8 from *Lutra maculicollis*.

DICUSSION: According to Skrjabin (1956) the genus *Baschkirovitrema* was erected by Skrjabin in 1944 to accommodate a single species, *Echinostomum incrassatum* (Diesing, 1850) Stossich, 1892. In the generic diagnosis given by Skrjabin (1956) the head spines are described as being arranged in a single row. In the present material the spines of the lateral series do not alternate but the spines of the dorsal group are arranged in a double row.

Braun (1901) described *incrassatum* Diesing, 1850 from *Lutra brasiliensis* and this description has been reproduced by Skrjabin (1956). The present material agrees with that described by Braun (1901) except that the head spines are larger (Table 1). From the figure given by Braun (1901) it would appear that the head spines were not all observed in profile and some of the measurements may have been carried out on spines that were foreshortened.

*B. incrassatum* has not previously been recorded from *Aonyx capensis* or *Lutra maculicollis* and is a new record for Southern Rhodesia.

## SUMMARY

A new Strigeid, *Prudhoella rhodesiensis* n.gen., n.sp., from Rhodesian otters

is described. The validity of the subfamily Alariinae Hall and Wigdor, 1918 is discussed.

Redescriptions of *Cynodiplostomum namrui* Kuntz and Chandler, 1956 and *Baschkirovitrema incrassatum* (Diesing, 1850) Skrjabin, 1944 are given.

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## The Systematic Position of the Genus *Dihemistephanus* Looss, 1901 (Trematoda: Digenea), with the Redescription of *D. lydiae* (Stossich, 1896) from the South Pacific*

Lewis E. Peters**

Looss (1901) erected the genus *Dihemistephanus* to include *D. lydiae* from the ocean sunfish, *Mola mola*, in European waters. Stossich (1896) had regarded the trematode as an echinostome and Looss mentioned its resemblance to *Echinostoma* and *Stephanochasmus* (=*Stephanostomum*). Poche (1926) assigned *Dihemistephanus* to the Family Acanthocolpidae, where it has since remained.

Little (1930) described as *Dihemistephanus sturionis* a trematode from the sturgeon, *Acipenser sturio*, and characterized the genus, but more on the basis of *D. sturionis* than the type, *D. lydiae*, as pointed out by Cable (1952). Because *D. sturionis* resembled certain other trematodes from sturgeons more than *D. lydiae*, Cable erected for *D. sturionis* the genus *Pristicola* and assigned it to the Subfamily Deropristiinae which had been proposed for the Family Lepocreadiidae by Cable and Hunninen (1942). Caballero (1950) transferred *D. brachyderus* Manter to the genus *Manteria*, leaving *D. lydiae* as the only species of *Dihemistephanus*. The writer's observations on that species show that Yamaguti (1958) was in error in making *Manteria* a subgenus of *Dihemistephanus*.

*A contribution from the Department of Biological Sciences, Purdue University, Lafayette, Indiana. Part of a Ph.D. thesis prepared under the direction of Professor R. M. Cable.

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Looss (1901) figured only the anterior end of *D. lydiae* but described other features that provide positive identification of its genus. Especially significant is a peculiar lobe overhanging the genital pore and of a type reported for no other trematode. In translation, he described an "oblique pad-like thickening of the cuticle . . . (with) a quantity of rod-like structures with their points appearing a little out of the cuticle, which remind one of head spines in their form and appearance. Whether they actually represent spine formations or something else I was not able to determine . . ." Looss' description leaves no doubt that precisely the same type of lobe occurs in specimens collected from *Mola mola* by Mr. R. V. Brundson, Victoria University, Wellington, N. Z. They were given to Prof. H. W. Manter who kindly provided the writer with 10 stained wholemounts and permitted two to be sectioned. As given in a preliminary abstract (Peters, 1958), it is evident from this material that *Dihemistephanus* is not an acanthocolpid. Following is a revised diagnosis of the genus:

*Dihemistephanus* Looss, 1901, *char. emend.*

Distomes with prominent cuticular spines, lateral and dorsal ones in a few rows near anterior end enlarged but not forming a distinct collar or corona; ventral lip of mouth with smaller spines separated from enlarged ones on each side and followed by a short zone of very small spines or none. Cercarial eyespot pigment diffuse. Prepharynx, pharynx and esophagus present; intestinal bifurcation well anterior to ventral sucker, ceca end blindly near posterior end of body. Genital pore median to submedian, close to anterior margin of ventral sucker, with overhanging lobe bearing cuticular pits containing slender structures resembling sensory processes. Cirrus sac with smooth (?) or tuberculated cirrus, prominent subspherical pars prostatica and internal seminal vesicle; external seminal vesicle and prostate cells posterior to cirrus sac; prostatic ducts converge at neck between seminal vesicles to enter cirrus sac, pass around internal seminal vesicle and join pars prostatica. Testes two, tandem, in posterior region of body. Ovary pretesticular, median or submedian; seminal receptacle large, Laurer's canal present. Vitellaria extensive, with small follicles in lateral fields throughout hindbody, sometimes extending a short distance into forebody. Uterus intercecal, between anterior testis or ovary and genital pore; metraterm prominent. Eggs numerous, of medium size, without filaments. Excretory vesicle tubular to saccate, with pore near posterior end of body. Type and only species, *Dihemistephanus lydiae* (Stossich, 1896) Looss, 1901, from the ocean sunfish, *Mola mola*.

Although the New Zealand material is undoubtedly congeneric with *D. lydiae*, it differs from that species as described by Looss (1901) in having a much longer cirrus sac and a tuberculated rather than smooth cirrus. However, Dollfus (personal communication) has obtained from Prof. Manter part of the material studied by the writer and has found that it is in agreement with specimens of *D. lydiae* from the coast of France. The occurrence of that species thus may well correspond with the range of *Mola mola*, a widely distributed oceanic fish. The following description is based on the New Zealand material (all measurements are in millimeters):

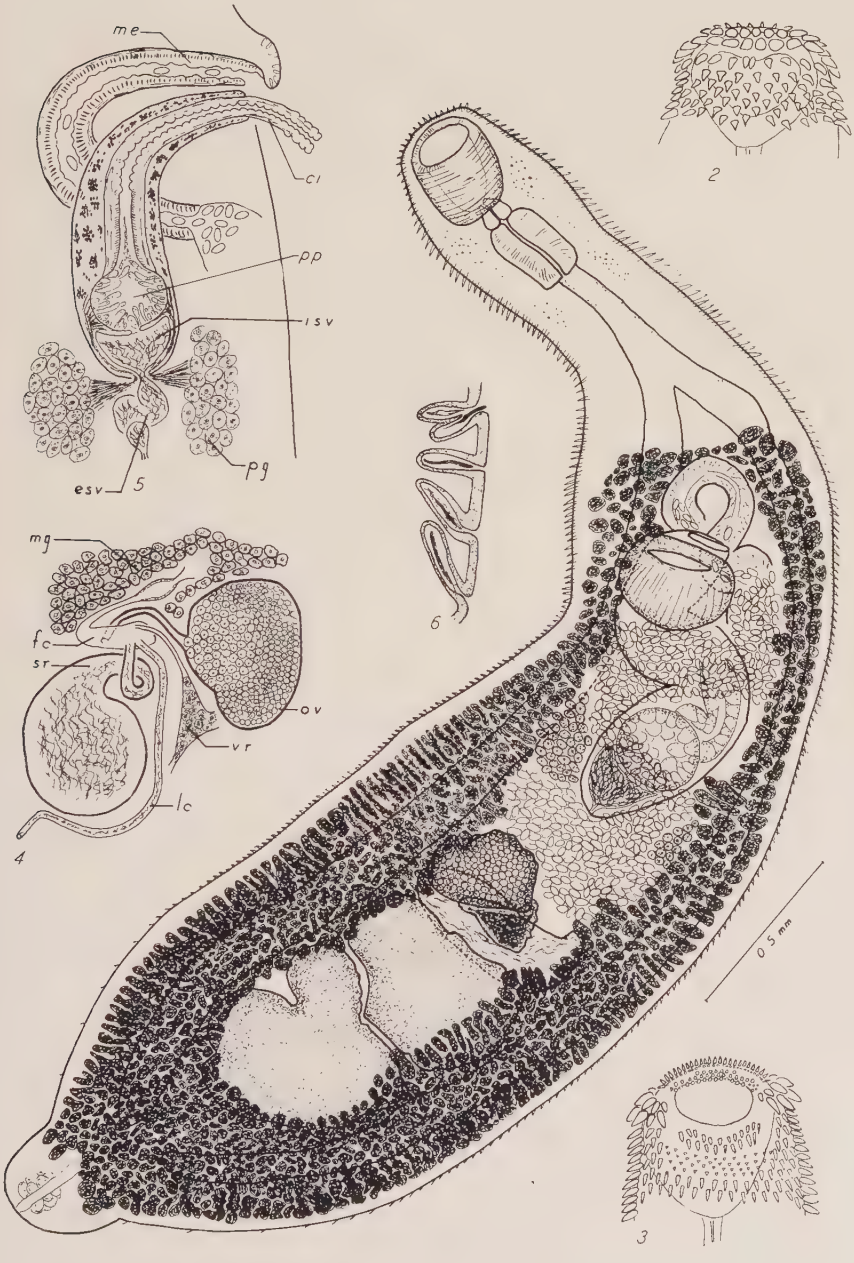
*Dihemistephanus lydiae* (Figures 1-6)

With the characters of the genus as emended above. Body 3.75-5.20 long, 0.83-1.04 in maximum width at about level of hindbody. Anterior end bluntly rounded to pointed; forebody narrow, 1.11-1.50 long. Cuticular spines gradually diminishing in size and number posteriorly, a few scattered almost to posterior end; spines at midlevel of forebody about 0.040 long, decreasing somewhat in size anteriorly but enlarging again at level of oral sucker to form an indistinct corona of two or three rows interrupted ventrally; dorsal lip of oral sucker with several rows of very small spines, ventral lip with about 18 spines in 2 indistinct rows followed by a zone of very small spines and then the abruptly enlarged ones of the cervical region. Ventral sucker 0.21-0.29 by 0.25-0.34, its lining with small spines. Oral sucker subterminal, spherical to pyriform, 0.23-0.27 by 0.23-0.30. Prepharynx 0.04-0.21 long; pharynx cylindrical, 0.20-0.26 by 0.13-0.18; esophagus 0.17-0.43 long; ceca smooth or slightly indented near bifurcation. Excretory bladder tubular, extending to midlevel of external seminal vesicle, dorsal and displaced somewhat laterally in region of testes; its epithelial lining with small projections, especially in posterior region. Excretory pore in small posterodorsal invagination, from which a narrow muscular duct with sphincter extends to bladder. Genital pore slightly sinistral, a transverse slit when closed, its overhanging lobe with structure in sagittal section shown in Figure 6; genital atrium short. Testes in posterior two-fifths of body, smooth to moderately indented; anterior testis 0.32-0.53 by 0.36-0.65, posterior 0.47-0.69 by 0.33-0.61. External seminal vesicle sinuous, tubular, covered dorsally and ventrally by numerous prostate cells; cirrus sac 0.73-1.07 by 0.26-0.30, ending about midway between ventral sucker and ovary or distinctly posterior to that level; internal seminal vesicle short and wide, concave anteriorly at junction with pars prostatica; ejaculatory duct long, cirrus with blunt papillae. Ovary postequatorial, to right of midline, entire or slightly indented, 0.16-0.33 in diameter. Oviduct enlarges to form fertilization chamber into which seminal receptacle, Laurer's canal, and vitelline reservoir open separately. Seminal receptacle dorsal, overlapping ovary and anterior testis. Pore of Laurer's canal on dorsal surface somewhat to left of midline near posterior edge of seminal receptacle. Vitelline reservoir between ovary and seminal receptacle; vitelline follicles in lateral fields overlapping ceca dorsally and ventrally from near posterior end of body almost to intestinal bifurcation, confluent posterior to testes and anterior to genital pore. Mehlis' gland well developed. Uterine coils fill most of intercecal space from ovarian level almost to genital pore; metraterm loops to right anterior to genital pore, thus entering forebody before joining genital atrium. Eggs numerous, undeveloped, 0.063-0.077 by 0.031-0.046.

PLATE I

*Dihemistephanus lydiae*. Figures 1-3, 6 drawn by microprojection; 4-5 by reconstruction from sections. Abbreviations: *ci*, cirrus; *esv*, external seminal vesicle; *fc*, fertilization chamber; *isv*, internal seminal vesicle; *lc*, Laurer's canal; *me*, metraterm; *mg*, Mehlis' gland; *ov*, ovary; *pg*, prostrate gland; *pp*, pars prostatica; *sr*, seminal receptacle; *vr*, vitelline reservoir.

Fig. 1. Hypotypo, ventral view.
Fig. 2. Anterior end, dorsal view.
Fig. 3. Same, ventral view.
Fig. 4. Female complex.
Fig. 5. Terminal genitalia.
Fig. 6. Lobe overhanging genital pore, sagittal section.


me
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vr
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4
6
2
3
0.5 mm

Hypotype: whole mount and sectioned specimen, No. 39418, Helminth. Coll., U. S. Nat. Mus.

According to Dollfus (personal communication), *Stenocollum fragile* (Linton, 1900) and an adult trematode included with some acanthocolpid metacercariae under *Stephanostomum valde-inflatum* (Stossich) by Linton (1940) are the same as *Dihemistephanus lydiae*. That material is from *Mola mola* but otherwise is too poorly known to be positively identified.

Two features of *Dihemistephanus* are of special significance to its family status: the independent rather than uterine seminal receptacle; and the external seminal vesicle and prostate cells. They are not characters of the Acanthocolpidae, the family to which the genus has heretofore been assigned, but instead occur commonly in trematodes of the Family Lepocreadiidae. Furthermore, the bulbous pars prostatica, diffuse eyespot pigment and elongated excretory vesicle are more like the lepocreadiids than the acanthocolpids. The genus *Dihemistephanus* accordingly is transferred to the Subfamily Lepocreadiinae of the Family Lepocreadiidae as defined by Cable and Hunninen (1942).

## Summary

Diagnosis of the genus *Dihemistephanus* is emended and *D. lydiae,* the type and only species, is redescribed from New Zealand material. Because it has an independent seminal receptacle, external seminal vesicle and other features that exclude it from the Acanthocolpidae, the genus is transferred to the Subfamily Lepocreadiinae, Family Lepocreadiidae.

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# Some Chemotherapeutic Trials in Canine Demodectic Mange

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Demodectic mange is one of the more difficult problems with which pet owners and small animal practitioners must contend. Treatment is tedious and results are frequently disappointing. Despite a wide selection of agents for specific medication, none is an established, reliable, or certain cure. Among materials that have been used with some success are lindane, chlordane, benzyl benzoate, rotenone, trypan blue, and chaulmoogra oil. With the exception of trypan blue, which is given by intravenous injection, these agents are applied topically or by wash once or twice weekly for 3 or 4 applications, or for several weeks if necessary.

Successes with certain systemic agents against cattle grubs led logically to the testing of these agents in other parasitisms, including demodectic mange. Gaafar and McDonald (1957) reported successful treatment of 8 clinical cases with a formulation containing 3 percent ronnel, applied twice weekly by wash for 6 to 8 weeks. There was complete remission of lesions and no mites could be demonstrated in skin scrapings. Later examinations, however, apparently were not made. Koutz (1957), on the other hand, obtained unsatisfactory results from the systemic use of ronnel. Dogs were given 100 mg./kg. of body weight orally and bathed, concurrently, in a 2 percent emulsion of the chemical. The regimen was followed twice a week for 10 weeks. Improvement was noted at the end of the treatment period although some lesions persisted, with eventual recurrence to approximately pre-treatment levels within 8 months. Sanger (1958) reportedly cured one dog with combined systemic (6 orally) and topical treatment (7 applications of 5 percent solution) followed by two applications of selenium sulfate.

These reports, together with unpublished experiences related to us by other workers, prompted us to summarize our findings in the experimental treatment of *Demodex canis* with ronnel,* Bayer 21/199**, dimethoate†, and Conteben, Bayer††, an unrelated chemical reported by da Graña and De Marzoratti (1955) and Guilhon and Petit (1958) to have action against this parasite. The results of our trials are the basis for this report.

## PROCEDURE

The hair coat of all dogs was clipped as closely as possible before treatment was initiated. This was done to allow closer inspection of the lesions, to evaluate better the progress under treatment, and, in topical applications, to facilitate medication and to conserve materials. Animals receiving the topical applications were given a soap and water bath, rinsed thoroughly, and dried. As a precaution against irritation from materials applied by wash, mineral oil was instilled into the conjunctival sac. Oral doses were given in hard gelatin capsules, and dimethoate (50 percent parenteral solution) was given

*Ronnel (0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate) [Dow ET-57]; Dow Chemical Company, Midland, Michigan.

**Bayer 21/199 (3-chloro-4-methylumbelliferone 0,0-diethylthiophosphate) [Co-Ral]; Chemagro Corporation, New York, New York.

†Dimethoate (0,0-dimethyl S-(N-methylcarbamoyl-methyl) phosphorodithioate) [American Cyanamid CL 12880]; American Cyanamid Company, Pearl River, New York.

††Conteben, Bayer (4-acetylaminobenzal thiosemicarbazone) [Tibione]; Schenley Laboratories, Inc., New York, New York.

intramuscularly. The animals were maintained, for the most part, on a balanced ration of commercial dog pellets; this was augmented occasionally with fresh meat and Liprotein (Upjohn), a commercial dietary supplement. All animals were confined in individual pens throughout the period of treatment. After apparent clinical recovery, however, four litter mate terriers occupied a common run before recurrence of lesions was noted.

## RESULTS

The results are summarized in table 1.

RONNEL: The first trial was with dog 726, a six-months-old male terrior, on October 29, 1956. After preparation for treatment, the dog was bathed in a 0.25 percent aqueous suspension prepared from a 25 percent wettable powder. Lesions were thoroughly massaged with the wash; and after treatment was completed, the dog was dried and returned to its pen. This regimen was repeated weekly for 3 weeks, but there was no apparent improvement. Consequently, the concentration of the wash was increased to 2 percent and the external medication augmented with concomitant, weekly, oral doses of the chemical at the rate of 100 mg./kg. of body weight. This regimen was followed for 3 additional weeks; at the end of this period, lesions were healed and no mites could be demonstrated. The animal was then given 2 weekly baths with a proprietary selenium preparation*** and, one week later, a thorough massage with olive oil. This dog has remained free of lesions and mites for more than 2 years.

Three litter mate terrier pups with marked and extensive lesions of demodectic mange were used for additional tests. Because of the favorable response in the previous trial, it seemed desirable to confirm the result and to investigate, in a limited way, the mode of action of the chemical. In one case the chemical was given orally, in another by wash, and in a third by both routes and in similar amounts. Treatment was repeated at weekly intervals.

Dog 748 was given 100 mg./kg. of body weight orally, but there was no improvement after 3 doses. The lesions, in fact, became progressively more marked and the mites more plentiful. The oral dosing was then supplemented by washing in a 2 percent aqueous suspension of the chemical, and this regimen was continued for 8 consecutive weeks. There was progressive improvement with remission of lesions and disappearance of mites; a relapse occurred, however, 5 months later.

Dog 747 was given a 2 percent aqueous wash for 6 successive weeks. There was remission of lesions and disappearance of mites at the end of this period. Eight months later, however, lesions and mites reappeared on this animal.

Dog 746 (Fig. 1) was given internal and external medication concomitantly and showed gradual improvement with remission of lesions and disappearance of mites by the end of the sixth week. Lesions and mites also reappeared on this animal, however, after a lapse of 3 months.

In a subsequent trial, this animal was washed with 2 percent ronnel (prepared from a purified form of the chemical) in a vehicle (emulsifiable base) developed by Du Toit and Fiedler (1956) for use in the treatment of nose bots of sheep. The formulation produced an intense dermal reaction, however, and the preparation was abandoned after 1 application. One week later, the 2 percent aqueous suspension described previously was applied twice weekly in conjunction with an oral dosage of 100 mg./kg. once a week. After

***Seleen (Abbott Laboratories, North Chicago, Ill.)

TABLE 1. Data on treatment of Demodex canis in dogs with ronnel, Bayer 21/199, dimethoate, and Conteben, Bayer.

| No. of Animal | Age (Mo.) | Chemical | Formulation | Treatment | | | | Results |
|---|---|---|---|---|---|---|---|---|
| | | | | Method | Dosage (mg./kg.) | Rate | Total (No.) | |
| 726 | 6 | Ronnel | 0.25% a.s. | Wash | | Weekly | 3 | No improvement |
| | | | 2.0% a.s. | Wash | | Weekly | | |
| | | | 25.0% w.p. | Capsule | 100 | Weekly | 3 | Apparent cure |
| 746 | 4 | Ronnel | 2.0% a.s. | Wash | | Weekly | 6 | Apparent cure; |
| | | | 25.0% w.p. | Capsule | 100 | Weekly | 6 | relapse in 3 months |
| | 9 | Ronnel* | 2.0% emul. | Wash | | | 1 | Severe dermal reaction |
| | 9 | Ronnel | 2.0% a.s. | Wash | | Semiweekly | 12 | Apparent cure; |
| | | | 25.0% w.p. | Capsule | 100 | Weekly | 6 | relapse in 13 months |
| 747 | 4 | Ronnel | 2.0% a.s. | Wash | | Weekly | 6 | Apparent cure; relapse in 8 months |
| 748 | 4 | Ronnel | 25.0% w.p. | Capsule | 100 | Weekly | 3 | No improvement |
| | 5 | Ronnel | 2.0% a.s. | Wash | | Weekly | 8 | Apparent cure; |
| | | | 25.0% w.p. | Capsule | 100 | Weekly | 8 | relapse in 5 months |
| 753† | 5 | Ronnel | 2.0% emul. | Wash | | | 1 | Severe dermal reaction |
| | 6 | Ronnel | 2.0% a.s. | Wash | | Weekly | 6 | Apparent cure; |
| | | | 25.0% w.p. | Capsule | 100 | Weekly | 6 | relapse in 6 months |
| 795 | 12 | Ronnel | 2.0% a.s. | Wash | | Semiweekly | 11* | Apparent cure; |
| | | | 25.0% w.p. | Capsule | 100 | Semiweekly | 11 | relapse in 3 months |
| 749 | 4 | Conteben | | Capsule | 25 | Daily | 42 | No improvement |
| | 5 | Conteben | | Capsule | 25 | b.i.d. | 7 | No improvement |
| 806‡ | 17 | Bayer 21/199 | 0.5% a.s. | Wash | | Weekly | 3 | No improvement |
| | 18 | Bayer 21/199 | 0.75% a.s. | Wash | | Weekly | 3 | No improvement |
| | 19 | Bayer 21/199 | 1.0% a.s. | Wash | | Weekly | 3 | No improvement |
| 814§ | 20 | Dimethoate | 50.0% solution | i.m. | 25 | | 1 | No improvement |
| | 21 | Dimethoate | 50.0% solution | i.m. | 25 | Daily | 3 | No improvement |

† Used previously as dog 749.
‡ Used previously as dog 747.
§ Used previously as dog 806.
* See text.

a.s. = aqueous suspension
w.p. = wettable powder
i.m. = intramuscularly

b.i.d. = twice daily
emul. = emulsion

6 weeks on this regimen, there was a remission of lesions. The regrowth of hair and the tonus of skin were good, and the mangy odor had disappeared. Mites could not be demonstrated in scrapings. Skin lesions were again positive for mites, however, 13 months later.

Dog 753, a litter mate of 746, 747, and 748, was washed with an emulsion containing 2 percent ronnel (prepared from M-858*, a 25 percent emulsifiable solution). The formulation produced an intense dermal reaction, however, and was abandoned after 1 application. One week later, the dog was started on a weekly regimen of internal and external medication. The 2 percent aqueous suspension was applied by wash and a dose of 100 mg./kg. was given in capsules. After 6 weeks, there was remission of lesions and no mites could be demonstrated in skin scrapings. Mites and lesions recurred, however, 6 months later. This animal was used previously in trials with Conteben, Bayer (see dog 749).

Dog 795, a privately-owned, year-old, female German Shepherd with demodectic mange, was referred to the laboratory for experimental treatment. After the usual preparation, she was started on a twice-weekly regimen of 100 mg./kg. in capsules and 2 percent aqueous suspension applied by wash. The sixth treatment was skipped because of inclement weather. On the seventh treatment occasion, the dog was bathed in a proprietary selenium preparation because of an excessive accumulation of scurf. Treatment with ronnel was resumed the following week and continued for 3 weeks. The response was not encouraging, however, and no further treatment with ronnel was given. Because the scurf persisted, the dog was bathed twice with the selenium preparation and massaged once with olive oil during the next 2 weeks. Although healing of lesions was not complete at this time, no mites could be demonstrated in skin scrapings. The dog was given a final bath in the selenium preparation and returned to the owner.

About 8 months later, the owner reported that no lesions were evident and the dog was in excellent condition. A small lesion had appeared on the thorax about 5 months earlier, but it responded promptly to topical applications of a proprietary rotenone preparation and no further evidence of mange has been observed.

*Bayer 21/199.* This chemical was used unsuccessfully in 3 trials with dog 806. Aqueous suspensions of 0.5, 0.75, and 1.0 percent, respectively, were applied weekly by wash for successive 3-week periods. Lesions persisted and mites were demonstrable at the conclusion of each series of treatments. This animal had been used previously in trials with ronnel (see dog 747).

*Dimethoate.* This chemical (50 percent solution) was ineffective in 2 trials with dog 814. A single intramuscular injection at the rate of 25 mg./kg. of body weight had no apparent effect on mites or lesions during a posttreatment observation of 5 weeks. A similar dose was then given for 3 consecutive days; but after 1 week, mites were still demonstrable in scrapings from active lesions. This animal was used previously in trials with ronnel and Bayer 21/199 (see dogs 747 and 806).

*Conteben, Bayer.* This chemical showed no promise in trials with dog 749. A dosage of 25 mg./kg. was given in capsules daily for 42 consecutive days, but progressive development of lesions continued. A similar dosage was then given twice daily for 7 additional days. No improvement occurred, and skin scrapings revealed many, apparently normal, mites.

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*Dow Chemical Company.

## DISCUSSION

In most cases the animals used in these trials were suffering from severe, suppurative demodectic mange. Mites were plentiful and easily demonstrated (Fig. 2).

Ronnel was the only chemical that showed promise of effective action against the follicular mite. A 2 percent aqueous suspension, applied by wash at weekly or semiweekly intervals, usually resulted in remission of lesions and disappearance of mites after 3 to 8 weeks. At this time there was normal hair growth and skin tonus, and there was little or no characteristic mangy odor. Clinical recovery had apparently been effected. In most cases, however, lesions recurred and mites were again demonstrable 3 to 13 months later.

Oral doses alone were ineffective, and they did not appear to hasten recovery when given in conjunction with external applications of the 2 percent aqueous suspension. The latter was well tolerated, but emulsions containing a comparable concentration of the chemical produced marked dermal re-

Figure 1. Dog 746. A, before treatment with ronnel. B, three months later.

actions. It was not determined whether this reaction was ascribable solely to the vehicle or diluent, but it should be noted that Koutz (1957) apparently did not observe dermal reactions in dogs that were bathed in a 2 percent emulsion prepared from Trolene M-947, a 50 percent weight/weight emulsifiable formulation.

The favorable action of ronnel against *D. canis* in the trials described herein is in agreement with similar observations by other investigators—Gaafar and McDonald (1957) and Sanger (1958). The recurrence of lesions and mites in most cases, however, confirms similar findings by Koutz (1957) and shows the need for an adequate posttreatment observation period.

The organophosphorus compounds Bayer 21/199 and dimethoate showed no promise of effective action against follicular mites in our trials; and Conteben, Bayer, an unrelated semicarbazone, showed no action although given in doses substantially larger than those reported to be effective (*loc. cit.*).

## SUMMARY

A 2 percent aqueous suspension of ronnel, applied by wash at weekly or semiweekly intervals for 3 to 8 weeks, showed promise of effective action against *Demodex canis*. In most cases, however, there was recurrence of lesions and mites from 3 to 13 months later. Oral doses of the chemical were ineffective, and they showed no favorable influence on the course of treatment when given concurrently with the aqueous suspension.

Bayer 21/199, dimethoate, and Conteben, Bayer were ineffective in limited trials.

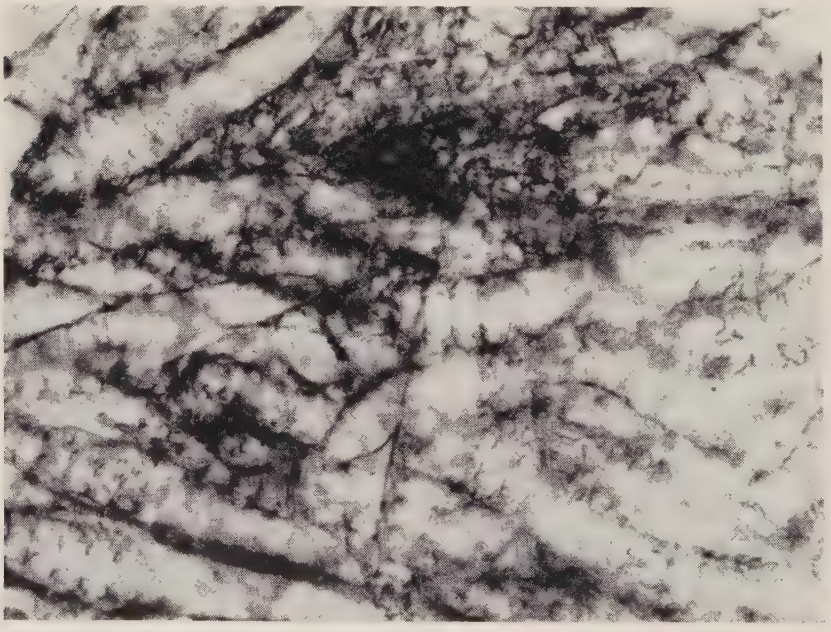

Figure 2. *Demodex canis* mites from skin scrapings of dog 746.

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## Three New Genera of Trematodes from Pacific Sea Serpents, *Laticauda colubrina* and *L. semifasciata**

WILLIAM H. COIL** and ROBERT E. KUNTZ***

The U. S. Naval Expedition to Lan Yü Island spent two weeks on this little-known island which lies approximately 45 miles east of the southern tip of Taiwan. This island carries a number of different names: Orchid Island, Botel Tobago, Koto-sho (Japanese) and Lan Yü (Chinese). The expedition was organized to permit medical studies of the Yami tribe of aborigines and to make investigations on the parasites of man and animals on the island. This is a continuation of the geomedical and biological studies by the U. S. Naval Medical Research Unit No. 2 on Taiwan and countries of southeast Asia.

The present paper is the first of a series based on the collection of helminths obtained by the examination of vertebrate hosts on Lan Yü in March of 1959.

Reptiles of the genus *Laticauda* spend time on land where they deposit their eggs. In the water, they are reported to feed on eels and other fish (Taylor, 1922). The hosts examined here were captured by the aborigines from holes in dead coral above the water line. One of us (R.E.K.) has observed that these snakes will move inland, especially at night, and they have been seen feeding along fresh-water streams in the New Hebrides and Solomon Islands. There can be no doubt that they have available a variety of animals which might serve as intermediate hosts of trematodes.

### MATERIALS AND METHODS

Most of the reptiles were captured alive and were examined soon after death. A few taken during the last two days on the island were returned alive to the laboratory in Taipei for examination. The viscera were removed and each system was examined separately with the aid of a dissecting microscope during and after maceration of tissue with splinter forceps and scissors. Additional examinations were made after tissues had been shaken in a capped bottle with several changes of fresh water.

*The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department. Studies from the Department of Zoology, No. 320.

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Trematodes were killed by quick immersion into hot water and then transferred to stender dishes with FAA (formalin-acetic acid-alcohol) for fixation. After 5 to 15 hours the helminths were transferred to vials with 70 percent alcohol plus 2 percent glycerine. Whole mounts were made by the use of Harris' haematoxylin or Semichon's carmine, terpineol, xylene, or methyl salicylate and piccolyte. All measurements are in millimeters.

The authors are indebted to G. M. Malakatis HM1, USN and James E. Reese HM1, USN of NAMRU 2 for assistance in procurement and examination of hosts. Dr. Robert F. Inger, Curator of Reptiles, Chicago Natural History Museum, has kindly provided identifications for the reptiles, Mr. E. S. Robinson helped ink some of the drawings.

## Family Acanthocolpidae Luhe, 1909

Two trematodes were found parasitizing the sea snake, *Laticauda colubrina,* collected on Lan Yü Island. Both specimens are mature; the one has only a few eggs and the other has several. In both, the cuticular spines appear to be intact; however, in one it is plain from the condition of the tissues that the animal is not in the best condition. Both specimens were sufficient for study.

This trematode fits best in the Acanthocolpinae Luhe, 1906, but it differs significantly from the genera *Acanthocolpus* Lühe, 1906 and *Tormopsolus* Poche, 1926.

### *Ophiotreminoides,* n. gen.

Diagnosis: with the characters of the family Acanthocolpidae Lühe, 1909. Body short, compact, covered with spines. Oral sucker terminal. Cephalic or oral spines absent. Prepharynx long and pharynx large. Esophagus very short. Ceca extend to posterior end where they join bladder to form uroproct. Acetabulum well-developed in anterior half of body. Testes diagonal in posterior half of body. Cirrus sac small, elongate, extending past posterior margin of acetabulum. Small internal seminal vesicle present. Spines not observed on cirrus. Ovary lateral, located about at midbody. No seminal receptacle observed. Uterine coils preovarian. Metraterm heavy, spines not apparent. Eggs comparatively large and thin-shelled. Vitelline follicles fine, extending from level of pharynx to posterior end, in lateral fields, dorsal to ceca, testes and ovary. Excretory bladder probably Y-shaped.

There are several features which this species holds in common with other acanthocolpids; however, there are several anatomical differences the possession of which require this group be relegated to generic status. The most similar genus to this one is *Tormopsolus* Poche, 1926, but our specimens differ by possessing an aspinose metraterm and cirrus, testes which are diagonal, a body which is compact rather than elongate, and possibly a different host group. None of these characteristics alone would serve to give its possessor generic rank, but collectively they set this group apart.

### *Ophiotreminoides orientalis,* n. sp.

Diagnosis: with the characters of the genus. Distomes of moderate length, 1.2-1.4, cuticle with heavy spines almost to posterior end, without collar spines. Body shape plump or rounded, not elongate. Width 0.41-0.7 at midbody. Oral sucker terminal, 0.14-0.16 wide. Prepharynx 0.085-0.1 long. Pharynx powerful, 0.10-0.13 wide, with characteristic shape. Esophagus absent or

very short. Ceca extend around lateral margins of gonads and join the excretory bladder posteriorly and form uroproct. Acetabulum larger than oral sucker, 0.18-0.19 wide, located 0.30 from oral sucker in anterior half of worm. Testes with slightly irregular margin, 0.29-0.34 long arranged in diagonal fashion in posterior half of body. Ovary spherical, lateral, just posterior to midpoint of worm, 0.102 wide. Vitellaria composed of numerous, small follicles distributed through dorsolateral field from level of pharynx to posterior end. Uterus with very few eggs located near end of cirrus sac, mediad to ovary. Genital pore large, just anterior to acetabulum. Cirrus sac long, extending past posterior margin of acetabulum. Internal seminal vesicle present. Metraterm present. Eggs 0.060 long by 0.042-0.045 wide.

HOST: *Laticauda colubrina* (sea snake).

HABITAT: Small intestine.

LOCALITY: Yeh Yu (a small aborigine village on Lan Yü Island).

SPECIMENS: Holotype in the Helminthological Collection of the U.S.N.M., No. 39412.

FAMILY ACANTHOSTOMIDAE POCHE, 1926

Twelve individual trematodes were recovered from three hosts (*Laticauda semifasciata*). All except two of these specimens were in good condition and made excellent whole mounts. It was apparent from a study of these worms that they could not be assigned to any existing genus due to their various peculiarities. Our specimens possess characters which are most similar to those of the genus *Acanthostomum* Looss, 1899.

*Ateuchocephala,* n. gen.

DIAGNOSIS: with the characters of the family Acanthostomidae Poche, 1926. Body moderately elongate. Cuticle aspinose. Anterior end lacking crown of spines. Oral sucker terminal. Prepharynx lacking and pharynx adjacent to oral sucker. Esophagus present. Caeca bifurcate between oral sucker and acetabulum and extend to posterior end where they open to exterior through separate, small pores. Acetabulum relatively large, about one-fourth body length from oral sucker. Testes in tandem in posterior quarter of body. Seminal vesicle extremely long, tubular, convoluted. No copulatory organ present. Genital pore adjacent and anterior to acetabuulm. Ductus hermaphroditicus short and thin-walled. Ovary slightly lateral just anterior to testes and ventral to seminal receptacle. Uterus preovarian with numerous eggs, extending into extracecal space. Vitelline follicles in posterior half of body located in field with some follicles in region of gonads. Excretory vesicle Y-shaped with a long stem, bifurcating at level of caecal bifurcation. Rami reach to level of oral sucker. Usually a parasite of marine snake.

Two genera of acanthostomids have been reported from reptiles: *Caimanicola* Freitas and Lent, 1938 and *Acanthostomum* Looss, 1899. It is this latter genus that our worms resemble most; however, there are several differences which make it mandatory to establish a separate category. The most striking of these differences is the absence of a crown of spines around the anterior end. In the event that cephalic or oral spines were present, which seems unlikely since our specimens were in good shape, there are a number of other differences which are sufficient to require the erection of a new genus. These are; 1) an aspinose cuticle, 2) prepharynx lacking, 3) caeca open to exterior some distance from posterior end, 4) uterus extends into extracaecal field, 5) the excretory bladder bifurcates in the region of the bifurcation of the gut.

*Ateuchocephala marinus*, n. sp. (Figs. 2 and 4)

DIAGNOSIS: with the characters of the genus. Distomes of moderate size with an aspinose cuticle. Cuticle somewhat wrinkled or folded. Body up to 4.9 long and up to 0.71 at the widest part. Oral sucker slightly subterminal, 0.22 to 0.31 wide. Prepharynx absent. Pharynx 0.10 to 0.12 wide, located on dorsal, posterior surface of oral sucker. Esophagus 0.20 to 0.32 long. Ceca long, extending near posterior end, 0.17 to 0.25, where they open to the exterior through small ani. Walls of ceca of moderate thickness. Testes frequently of irregular shape, usually touching or very close together, 0.22 to 0.29 long, located in posterior quarter of body. Cirrus sac lacking. Genital pore just anterior to acetabulum. Ductus hermaphroditicus extends from genital pore to a point dorsal to acetabulum where male and female ducts join. Numerous gland cells not apparent in this region. Long, sinuous seminal vesicle extends from region of acetabulum a short distance posteriorly. Ovary 0.17 to 0.24 wide, ventral, and a short distance anterior to testis. Seminal receptacle ellipsoidal, dorsal to ovary. Loops of uterus extend from ovary to region of acetabulum overlapping the ceca and dorsal to them. Uterus joins ductus hermaphroditicus dorsal to acetabulum. Excretory bladder a single stem to level of gut bifurcation where it divides and rami extend to level of pharynx and oral sucker. Numerous pigment granules in dorsal field in region slightly anterior to acetabulum. Vitelline follicles mainly lateral, but extending mediad in region of testes, located in posterior two-fifths of body extending almost to ani. Eggs 0.015 to 0.017 by 0.026 to 0.030.

HOST: *Laticauda semifasciata* (sea snake).

HABITAT: Small intestine.

LOCALITY: Hung Tou and Tung Ching (Villages on Lan Yü Island).

TYPE SPECIMENS: In the Helminthological Collection of the U.S.N.M., No. 39414.

FAMILY HEMIURIDAE LUHE, 1901

A large number of specimens was recovered from the lungs of sea snakes collected on the west coast of Lan Yü Island. Eight of thirteen hosts were infected. Parasites in this family are generally found in the intestines of fishes, and it is highly unusual to find them in the lungs of reptiles. There can be no doubt, however, that these worms are in their natural host when one considers the fact that they infest the lungs and that they were found in 8 individual hosts.

*Pulmovermis*, n. gen.

DIAGNOSIS: with the characters of the family Hemiuridae Lühe, 1901. Body cylindrical, aspinose, with small ecsoma. Oral sucker subterminal. Prepharynx absent. Esophagus absent or very short. Ceca bifurcate at pharynx and extend to posterior extremity of body. Acetabulum larger than, and located close to, oral sucker. Testes in tandem in posterior half of worm. Seminal vesicle tubular and with a heavy muscular wall extending in a convoluted fashion from the testes to the acetabulum. Ductus hermaphroditicus enclosed by a muscular layer. Genital pore just behind pharynx. Ovary in posterior quarter of body posterior to testes. Vitelline gland just posterior to ovary, divided into seven unequal lobes. Uterus with many eggs extending to posterior region. Excretory rami with a commissure dorsal to oral sucker. Parasites in lungs of marine snakes.

These trematodes possess the typical hemiurid structures, but have to be placed in a new genus due to the possession of a combination of characters unknown among the genera now assigned to the Hysterolecithinae. They are

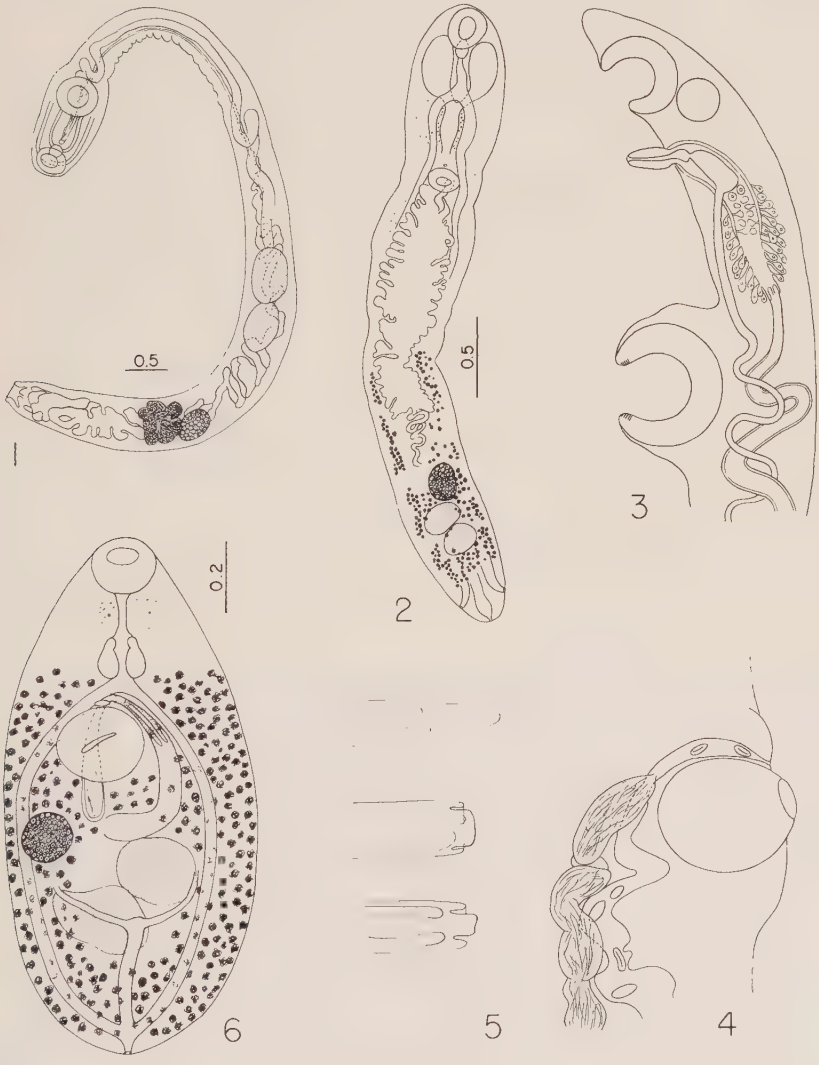


Fig. 1. *Pulmovermis cyanovitellosus,* n. gen., n. sp., ventral view (Hemiuridae).

Fig. 2. *Ateuchocephala marinus,* n. gen., n. sp., ventral view (Acanthostomidae).

Fig. 3. *Pulmovermis cyanovitellosus,* n. gen., n. sp., freehand sketch from sections.

Fig. 4. *Ateuchocephala marinus,* n. gen., n. sp., lateral view drawn with the aid of a camera lucida.

Fig. 5. *Pulmovermis cyanovitellosus,* n. gen., n. sp., sketches of posterior end showing various positions of the ecsoma.

Fig. 6. *Ophiotreminoides orientalis,* n. gen., n. sp., ventral view (Acanthocolpidae).

most similar to the genus *Hysterolecitha* Linton, 1910. The trematodes studied here are different from existing genera by: 1) a very elongate body shape; 2) testes in tandem in the posterior half of the body; 3) absence of an esophagus; 4) the placement of the acetabulum in close proximity to the oral sucker.

*Pulmovermis cyanovitellosus,* n. sp. (Figs. 1, 3, and 5)

DIAGNOSIS: with the characters of the genus. Large, elongate distomes, with a cylindrical body. Ecsoma present, but excretory pore subterminal. Body length up to 17 mm. (in alcohol) and width at level of acetabulum up to 1.2. Eggs present in specimens as short as 5 mm. Cuticle without spines, but papillated. Oral sucker subterminal, 0.29-0.53 long. Prepharynx absent. Pharynx 0.20-0.30 long. Esophagus absent. Ceca extend to posterior extremity. Gut with villis-like folds and projections. Acetabulum larger than oral sucker, 0.44-0.73 long, located 0.49-0.63 from oral sucker. Testes smooth elongate, in posterior half of body, in tandem, frequently in contact, more often separated, 0.58-0.97 long. Seminal vesicle long, tubular with heavy, muscular wall, up to 0.034 thick. Ovary subspherical in posterior quarter of body 0.38-0.58 wide. Vitellaria just posterior to ovary, divided into seven unequal lobes, 0.53-0.90 long, appear blue in alcoholic specimens in tungsten lamp light. Uterus extensive with numerous eggs, extending almost to posterior end. Eggs 0.017-0.023 by 0.010-0.012. Genital pore just posterior to pharynx. Ductus hermaphroditicus enclosed in muscular pouch open at proximal end, extensible for a short distance.

HOST: *Laticauda semifasciata* (sea snake).

HABITAT: Lungs.

LOCALITY: Yeh Yu.

SPECIMENS: Holotype and paratypes in the Helminthological Collection of the U.S.N.M., No. 39413.

Yamaguti (1933) reported two species from the sea snake *Laticauda laticauda* (*Harmotrema laticaudae* and *Oesophagicola laticaudae*).

It is of some interest to note the host-parasite relationships of the families of trematodes represented here. Hemiurids are generally found in the stomachs or gall bladders of marine fishes. The presence of several worms, in the lungs, in a number of individuals canont be considered accidental as might be the case if the worms were found in the intestine. It seems certain that this hemiurid was acquired through the reptile's long association with marine life. On the other hand representatives of the Acanthostomidae are found in both fishes and reptiles found associated with freshwater hosts. It would seem likely then that the parasite described here (*Ateuchocephala marinus*) is acquired in terrestrial or freshwater habitats. The third family represented here is the Acanthocolpidae; a group which is generally found in marine fishes. However, the presence here of only two barely mature worms in the sea snake's intestine proves nothing conclusive. It is very possible the reptile might have eaten well-developed metacercariae a few days before the examination.

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# A Comparison of *Leucocytozoon simondi* in Pekin and Muscovy Ducklings*

N. THEODORE BRIGGS**

The genus *Leucocytozoon,* in common with other genera of Hemoproteidae, is characterized by the fact that shizogony is confined to the internal organs of the vertebrate host, and the only obvious evidences of this process in the peripheral circulation are the gametocytes; the absence of malaria-like pigment in the gametocytes or the infected cell separates this genus from other Hemoproteidae. In summarizing his own and earlier studies on this "malaria-like" disease of ducks, O'Roke (1934) outlined the life cycle and incriminated black flies (Family Simuliidae) as vectors of this sporozoan. Chernin's studies (1952a, 1952b, 1952c) have further characterized the infection in pekin ducks in Northern Michigan. Ducks surviving the primary infection show gametocytes of two types, round gametocytes found in enlarged rounded host cells and elongate gametocytes found in enlarged fusiform host cells. The phenomenon of gametocytic "dimorphism" has been discussed by Huff (1942), Cook (1954), Fallis, Davies, and Vickers (1951), and Otto (1958), but no completely satisfactory explanation has resulted from the information presently available.

It is generally agreed that *Leucocytozoon* infections are restricted to avian hosts and Coatney (1937) has compiled a host catalog for the genus which has been described from over 100 avian species. Manwell (1951) has discussed the problems associated with the taxonomy of this parasite which in most cases is based on a supposed but not demonstrated host specificity. The species found in ducks, *L. simondi,* was described by Mathis and Leger from the teal. Since then the host range has been extended to include a wide variety of wild ducks and Fallis, Pearson and Bennett (1954) suggested that geese may be included among its hosts. The latter workers, however, have presented experimental and epizootic evidence that there is some degree of host specificity for *L. simondi;* infections experimentally and naturally transmitted to domestic ducks were not acquired by ruffed grouse, chickens, turkeys, and pheasants, each of which has been reported previously to be parasitized by its own species of *Leucocytozoon.*

Although many species of *Leucocytozoon* have been described, the course of primary infections in the avian host have been reported in greatest detail for *L. simondi* in the pekin duck. It was of interest, therefore, to compare infections of *L. simondi* in muscovy ducks with those in pekin ducks from which most of our present knowledge of the vertebrate infections has come.

## MATERIALS AND METHODS

White pekin ducklings, *Anas platyrhynchos,* were purchased from a commercial hatchery and were kept in blackfly-proof quarters after introduction into the enzootic area. Muscovy ducklings, *Cairina moschata,* were obtained from a local breeder and isolated in the above-mentioned quarters within 24-48 hours after hatching. Blood smears made on control pekins maintained in these quarters failed to reveal any evidence of infection.

*From the University of Michigan Biological Station, Cheboygan, Michigan. These studies were supported by a grant from the Joseph Henry Fund of the National Academy of Sciences to Dr. G. F. Otto.

The author is indebted to Mr. Fred Otto for technical assistance during the course of this work.

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Preliminary work was done to determine the suitability of a farm site (in Cheboygan County, Michigan) and a lakeside site (at the University of Michigan Biological Station) for exposure of birds to natural infection. On June 29-30, 1953, approximately a dozen white pekins were exposed to natural infection at each of the two sites. Some ducklings were unpenned, but others were penned by enclosures either completely surrounded by chicken wire or with an open top. Eight days later all ducklings were blood positive. Following examination of these birds they were left at their respective sites as additional sources of infection for the experimental ducklings introduced into these sites at later dates.

The experimental ducklings were introduced into these environments when 2 to 3 weeks old, maintained there for 8 to 10 days, and then returned to the blackfly proof quarters for further observation. Thin smears were made from blood obtained from the leg vein of the ducklings on the 7th and 8th days of exposure and every 2-3 days thereafter. All slides were fixed in methyl alcohol and stained with Giemsa's stain. They were examined by the time-search method (Chernin, 1952a) under high power (430x) and the gametocytes counted in a 5 minute search. Separate totals were kept for round or elongate gametocytes.

Three groups of 8 to 9 muscovies and a similar number of pekins were thus exposed. Two groups were exposed during the anticipated height of the epizootic and the third group after the anticipated peak of transmission. Thus group A was exposed at the lakeside site for 8 days beginning July 18 (July 18-26). Half of each species was penned within the wire enclosure; and although the other half was turned loose, they congregated near the pen. Group B was exposed unpenned at the farm site for 10 days beginning July 16 (July 16-26). Group C was exposed in the farm site pen for 10 days beginning July 26 (July 26 to August 5).

## Results

The infection and mortality rates for the three groups are summarized in Table 1. That transmission did in fact vary among these groups is suggested by the percent of ducklings with patent infections, but even more clearly by the percent of ducklings with patent infections on the 8th day of exposure and by the mortality rates. Thus, all of the 17 ducklings in Group A developed parasitemia by the last day of exposure, day 8, and all died. In contrast, ducklings in Group B and C were less severely infected in spite of the fact that they were exposed to infection for an additional 2 days. Thus, only one bird in Group C developed a parasitemia by the 8th day of exposure, and only one died. That the severity of infection in Group B was intermediate between Groups A and C is suggested by the 100% patency but only 89 and 12% mortality for the two species.

Table 1 brings out even more sharply the apparent differences in the character of infection acquired by the two host species. Such host differences are not evident in the very heavy infections of Group A in which all ducklings of both species became rapidly infected and died within 18 days. In Group B, however, all of the pekins developed parasitemia within 8 days and 8 of the 9 died within 21 days; only 6 of the 8 parallel muscovies developed parasitemia within 8 days and only 1 died. Although all of the pekins of Group C became infected, only 4 of the 7 parallel muscovies developed patent infections, none within 8 days, and none died during the period of observation (25 days) which was four days longer than for group B.

Data on mean gametocyte levels and time of death for these same groups (Figure 1) again suggest a graded severity of infection. The pekins of Group C, which were exposed after the height of the epizootic, had the lowest parasitemias among the pekins. The one death was much later than in the other two groups. Round gametocytes in these ducklings reached a peak on day 14 and the elongate on day 17. In contrast, gametocytes reached higher levels in the pekin ducklings exposed at either of the two exposure sites during the height of the epizootic (Groups A and B). In Group B both round and elongate gametocyte peak counts exceeded 250 in 5 minutes search and the peaks occurred earlier than in Group C. In Group A the infection was so severe that all these pekins died by day 10, at or before the round gametocyte peak and before the appearance of any elongate forms.

Furthermore, Figure 1 clearly shows marked differences in the responses of the two hosts to the same exposure risk. In each of the three situations, parasitemias in the muscovies were conspicuously lower, and deaths generally occurred later than in the parallel pekins. Although the round gametocytes appeared at about the same time in the two host species under the heaviest exposure risk (Group A), there was some delay in the muscovy parasitemia following lighter exposure, particularly in Group C. Moreover, the numbers of elongate gametocytes were negligible in the muscovies of Groups A and B and were not seen at all in Group C. The pekins of all groups died with relatively high parasitemias, and all but one of these deaths appeared to be associated with a rising parasitemia. In contrast, the muscovy mortalities were associated with relatively low parasitemias, and six of these eight deaths occurred after the parasitemia had started to decline from even the low peak.

It should be noted at this point, however, that 5 muscovy losses occurred during the various exposure periods but are not included in either Table 1 or Figure 1. Two of these unpenned ducklings in Group A disappeared on the 6th day of exposure, and one in Group B disappeared between the 8th to 10th days of exposure. They were never found either dead or alive. All three of these muscovies showed small to moderate numbers of ring stages in red cells on slides made 1 to 2 days before they disappeared. In addition, two penned muscovies from Group C were found dead on the 10th day of exposure; examination on the eighth day of exposure had failed to reveal any parasitemia. In any event, there is no evidence that any of these five had a heavy parasitemia, and their exclusion from the tabular data does not significantly alter the observations made on comparative mortality rates and severity of infection in the two host species.

Table 1. Infection and mortality rates among white pekin and muscovy ducklings* exposed in parallel to natural infection with *Leucocytozoon simondi*.

| Group | Ducks Exposed | Days of Exposure | % Patent in 21-25 days | % Patent on Day 8 | % Mortality in 21-25 days |
|---|---|---|---|---|---|
| A | 10 white pekin | 8 | 100 | 100 | 100 |
| | 7 muscovy | 8 | 100 | 100 | 100 |
| B | 9 white pekin | 10 | 100 | 100 | 89 |
| | 8 muscovy | 10 | 100 | 75 | 12 |
| C | 8 white pekin | 10 | 100 | 12 | 12 |
| | 7 muscovy | 10 | 57 | 0 | 0 |

*Does not include 5 muscovies which died during the exposure periods.

## DISCUSSION

An examination of the gametocytemias and mortalities in parallel groups of white pekin and muscovy ducklings shows that the two hosts developed very different types of infections. Following relatively low exposure risks which allowed at least some of the infected pekins to survive, fewer muscovies became patent, they developed lower parasitemias, and suffered fewer deaths. Even when transmission was severe enough so that all exposed ducklings of both species became patent and died, muscovy deaths occurred relatively late, during the period of declining parasitemia, whereas pekin deaths were invariably associated with early fulminating parasitemia. Furthermore, muscovies appeared to show less correlation between parasitemia and the severity of disease than did pekins. Thus, the parasitemias in Group A muscovies, all of which died, were not appreciably higher than those in muscovies of Group B, in which only one of eight died.

Among the explanations for the difference in the gametocytemia in these two hosts is the possibility that the blackflies may feed more readily on pekins than on muscovies. Thus, under apparently identical exposure risk, the muscovies may actually receive fewer black fly bites and thus actually have a lower exposure risk than the pekins. However, since the muscovy fatalities are not only delayed but are associated with even lower parasitemias than seen in the surviving pekins, alternative explanations must be considered.

Since the deaths among the pekins were associated with rapid blood destruction near the peak of the parasitemia, it would appear that anemia was at least an important contributory factor, if not the primary cause of death among these pekins. In addition to the blood destruction, there is extensive destruction of liver and spleen (Fallis, Davies, and Vickers, 1951; Newberne, 1957), and other tissues (Fallis, Anderson, and Bennett, 1956) associated with the schizogonous cycle. Since the muscovy deaths are delayed and are not associated with as high a parasitemia as are pekin deaths, it appears that muscovy deaths may result from injury to the internal organs. This raises the question as to whether muscovies are disproportionately injured by a given amount of schizogony or whether the underlying schizogony is much more extensive than would be suspected from the rather nominal amount of gametocytemia. Thompson and Huff (1944) have reported that when the malaria of the Mexican lizard was transferred to a closely related but abnormal host, the collared lizard, predominantly exoerythrocytic stages developed in the latter with relatively few erythrocytic parasites. Even abnormal transfer within a normal host species, such as repeated passage of *Plasmodium gallinaceum* in chick embryoes (Haas, et. al., 1946) or within avian tissue culture (Lewert, 1950) has been shown to produce predominantly exoerythrocytic schizogony. It appears that the muscovy may be an abnormal host for *L. simondi,* and it would not be surprising that in such a host schizogony would be ineffective in gametocyte production. What, if any, additional depression of gametocytes might result from acquired immunity is unknown.

The present studies support the report by Chernin (1952a) that in individual pekins there is relatively little quantitative correlation between the number of round gametocytes and the subsequent number of elongate gametocytes. Nevertheless, on the average the two forms were present in about equal numbers in this host. In the muscovy, however, elongate gametocytes were rare and never accounted for more than five percent of the total number

of gametocytes. An adequate explanation of this must necessarily await more complete information of the essential differences between the two morphological variants than is now available. Cook (1954) has discussed at length possible explanations for this gametocytic "dimorphism" without coming to any satisfactory conclusion. The present findings do not selectively support any of the possibilities she presents but add an additional alternative to be considered, namely, that the host species may be a factor in the nature and extent

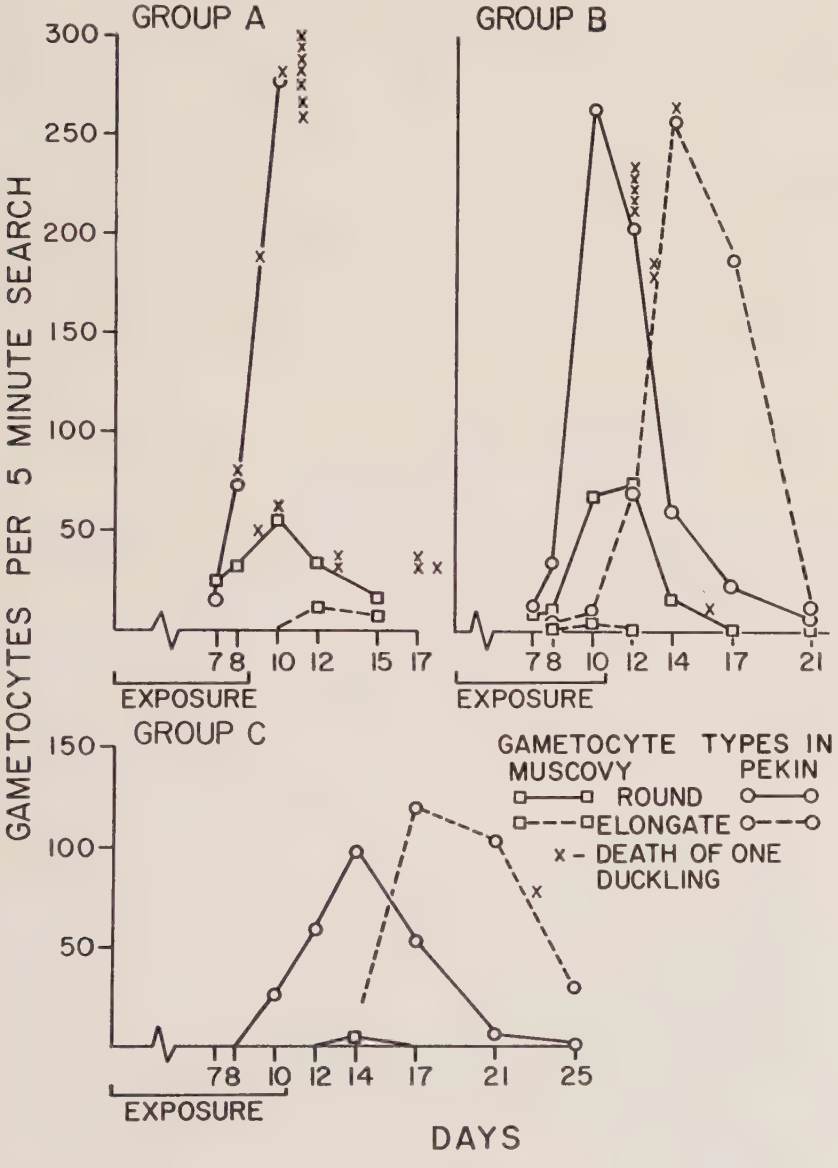


Figure 1. Mean gametocytemias in pekin and muscovy ducklings. Since the mean of the surviving ducklings is used, the number averaged is reduced by each death.

of the gametocytemia resulting from the underlying exoerythrocytic schizogony. It would appear that a more adequate study of the interrelationship of tissue and blood stages of *Leucocytozoon* in different, but closely related,

Zool. 29: 305-328.

host species might help elucidate the peculiarities in the life cycle of this sporozoan.

## Summary

Comparative gametocytemias and mortality rates are reported for white pekin and muscovy ducklings simultaneously exposed to natural infections with *Leucocytozoon simondi*. Although both hosts became readily infected, mortalities and gametocyte levels differed considerably among the various exposure groups. Muscovies, however, consistently had much lower gametocytemias than pekins, tended to develop gametocytemia later than the pekins, and had proportionately fewer elongate forms (never more than five percent of the total). Furthermore, the mortality rate was lower among the muscovies and deaths were delayed. It is not clear whether such differences between the infections in pekin and muscovy ducklings result from differences in susceptibility to *L. simondi* or differences in susceptibility to attack by black flies. In any event, deaths among the muscovies appear to result from exoerythrocytic schizogony rather than from blood destruction.

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# Effects on Calves of Gastrointestinal Nematodes Naturally Acquired

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The writers are unaware of any published information on the effects of helminth parasitism on weight gain and general well-being of calves based on the performance of animals with infections naturally acquired on contaminated pastures *versus* that of animals raised worm-free and maintained on clean pastures. The results of two small-scale experiments in which this comparison was made are reported in this paper.

## MATERIALS AND METHODS

A permanent pasture of about 2 acres, located at Beltsville, Maryland, originally planted to Kentucky bluegrass and clovers and not grazed for several years, was divided into two equal plots—A and B. At the beginning of each experiment, the quality and quantity of the forage on each plot was about equal. Plot A served as the clean pasture.

Between the fall of 1953 and the spring of 1954, plot B was moderately contaminated, mainly naturally, with cattle manure that contained an undetermined number of gastrointestinal nematode eggs. Later in the spring, from May 6 to June 15, 1954, manure that contained an estimated 438 million eggs was spread evenly over the plot. The number of eggs was estimated by the weight of the manure and counts of eggs in weighed samples.

Experiment 1 was started on June 23, 1954. Comparable calves, raised helminth-free and passing few oocysts of *Eimeria* spp., were used. Their ages ranged from 1.5 to 1.8 months when put out to graze. Two calves were placed on each plot and after 5 to 7 weeks, one calf of each group was replaced by another helminth-free calf (table 1). Thus, 6 calves were involved in the experiment. The experiment was concluded on October 13, 1954.

In preparation for experiment 2, forage samples from plot B were examined in the spring of 1958 and yielded only a few larvae per pound. Hence, cattle manure, which contained an estimated 289 million gastrointestinal nematode eggs, was spread evenly over the plot for a week in late July. This contamination was about the degree that would have been produced by 4 lightly- to moderately-infected yearlings grazing on the area for one month.

Experiment 2 was started on September 4, 1958. Three comparable test calves were placed on each plot. Those placed on plot A were raised helminth-free, except for a few *Strongyloides*. Two of those placed on plot B had acquired very slight infections with other worms before the experiment started. The ages of the calves ranged from 3.2 to 3.6 months when put out to graze. The experiment was concluded on November 20, 1958.

A plan of modified strip grazing was used in both experiments. The calves had access to the entire plot for only a part of each experiment. The management was exactly alike on plots A and B.

The calves were weighed each week during the experiments and the eggs per gram of feces were also determined weekly. The general appearance of the calves and the consistency of their feces were observed weekly or oftener.

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*Dr. Robert Rubin assisted with experiment 1.

The infected calves from plot B were necropsied on the day of death or removal from pasture, and the worm loads were determined as described by Goldberg and Rubin (1956). The control calves on the uncontaminated plot were not killed.

## RESULTS AND DISCUSSION

GENERAL RESULTS: As determined by fecal examination, in each experiment the calves on contaminated plot B promptly acquired a significant mixed infection of gastrointestinal nematodes. The peak count of eggs per gram averaged 2,100 and 947 in experiments 1 and 2, respectively.

The calves on the uncontaminated plot A remained worm-free or practically so. On one occasion in each experiment, the feces of one control calf contained 1 egg per gram besides a few *Strongyloides* eggs. None of the calves on plot A showed any signs of illness.

In experiment 1, calf 78 on contaminated plot B did not show clinical signs of disease. Calf 80 began to lose weight after 3 weeks and then sickened with a greenish, watery diarrhea, typical of several types of gastrointestinal helminth parasitism, and died on the 33rd day of the test. Its replacement, calf 71, survived, but it also developed a similar diarrhea toward the end of the experiment.

In experiment 2, calf 57 died after 9 weeks. Although it had gained almost no weight for a month before death, it was not diarrheic, and death may have been due primarily to bloat. Neither of its pasture mates showed clinical evidence of disease during the test.

AVERAGE DAILY WEIGHT GAIN: As determined from the data in table 1, in experiments 1 and 2 the average daily weight gains of the calves on the clean plot were respectively, 213 and 53.7 per cent greater than that of the corre-

TABLE 1.—Average daily weight gain (in pounds) of calves on contaminated and clean pastures

| Experiment no. | Calf no. | No. days on pasture | Initial weight | Gain or loss | Average daily gain or loss |
|---|---|---|---|---|---|
| | | *Contaminated Pasture* | | | |
| 1 | 80* | 34 | 171 | —29 | —0.853 |
| 1 | 71** | 77 | 150 | 22 | 0.286 |
| 1 | 78 | 112 | 160 | 74 | 0.661 |
| Total or average | | 223 | | 67 | 0.300 |
| 2 | 57*** | 63 | 150 | 33 | 0.524 |
| 2 | 47 | 77 | 217 | 48 | 0.623 |
| 2 | 58 | 77 | 168 | 32 | 0.416 |
| Total or average | | 217 | | 113 | 0.521 |
| Grand total or average | | 440 | | 180 | 0.409 |
| | | *Clean Pasture* | | | |
| 1 | 82 | 34 | 175 | 36 | 1.059 |
| 1 | 76**** | 62 | 155 | 70 | 1.129 |
| 1 | 79 | 112 | 153 | 89 | 0.795 |
| Total or average | | 208 | | 195 | 0.938 |
| 2 | 46 | 77 | 190 | 50 | 0.649 |
| 2 | 56 | 77 | 160 | 60 | 0.779 |
| 2 | 59 | 77 | 165 | 75 | 0.974 |
| Total or average | | 231 | | 185 | 0.801 |
| Grand total or average | | 439 | | 380 | 0.866 |

*Died of parasitism. **Replacement for No. 80. ***Died, probably from bloat. ****Replacement for No. 82 to match weight of No. 71.

TABLE 2.—Worms recovered from calves on contaminated plot B

| | Experiment 1 | | | Experiment 2 | | |
|---|---|---|---|---|---|---|
| Calf No. | 80 | 71 | 78 | 57 | 47 | 58 |
| Days on plot | 34 | 77 | 112 | 63 | 77 | 77 |
| Kinds of worms: | Number of worms* | | | | | |
| *Ostertagia ostertagi* | 9,280 | 9,900 | 5,020 | 7,000 | 4,375 | 4,700 |
| *Trichostrongylus axei* | 8,155 | 7,000 | 18,600 | 1,710 | 360 | 550 |
| *Haemonchus contortus*** | 0 | 552 | 187 | 32 | 26 | 4 |
| *Cooperia oncophora* | 31,535 | 0 | 9,965 | 10,860 | 6,125 | 5,965 |
| *Cooperia punctata* | 6,865 | 15,080 | 13,735 | 18,425 | 19,550 | 10,465 |
| *Cooperia* larvae | 1,750 | 300 | 3,700 | 7,435 | 4,000 | 4,390 |
| *Nematodirus helvetianus* | 7,365 | 0 | 2,020 | 49,550 | 27,065 | 20,775 |
| *Trichostrongylus colubriformis* | 3,025 | 1,425 | 7,090 | 0 | 0 | 0 |
| *Bunostomum phlebotomum* | 0 | 0 | 0 | 75 | 30 | 45 |
| *Moniezia* | 0 | 0 | 0 | 16 | 27 | 21 |
| *Oesophagostomum radiatum* | 5 | 8 | 27 | 3 | 16 | 16 |
| *Trichuris ovis* and *T. discolor* | 7 | 37 | 44 | 114 | 169 | 128 |
| Total | 67,987 | 34,302 | 60,388 | 95,220 | 61,743 | 47,059 |

*Both adults and larvae unless otherwise specified. ***Sensu lato*

sponding groups on the contaminated plot. In the two experiments combined, the average gain for all calves on the clean plot was approximately 112 per cent greater than that for all on the contaminated plot.

WORM LOADS: Table 2 shows the numbers and kinds of worms recovered at necropsy. The greatest number, about 95,000 was recovered from calf 57, which apparently died from causes other than parasitism. Calf 80, whose death was attributed to parasitism, and calves 78 and 47, despite the fact that they showed no clinical evidence of parasitic disease, yielded approximately equal numbers of worms, about 60,000 to 68,000. Fewest worms were recovered from calf 71, which did show signs of parasitic disease during the experiment.

These findings suggest than the development of severe or fatal helminthic disease in a calf is only approximately related to the number of worms harbored by the animal at a particular time. The development of the disease depends on a complex of factors which may include (1) the rate of intake of infective larvae; (2) the total larval intake; (3) the duration of infection; (4) the pathogenicity of the species; and (5) the sensitivity of the individual to the effects of the worms and its ability to counteract the effects.

## SUMMARY

Twelve calves, raised helminth-free or practically so, were used in two grazing experiments, the first lasting 3.6 and the second, 2.5 months. Half of the calves were maintained on a clean pasture and half on a pasture that was comparable, except that it had been recently contaminated with cattle manure containing gastrointestinal nematode eggs. The calves on the contaminated pasture became moderately infected with worms, but those on the clean pasture remained worm-free or practically so. The calves on the clean pasture gained weight twice as well on the average as those on the contaminated one, and they were in better condition.

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## The Influence of Carbon Dioxide on Respiration of Certain Plant-parasitic Nematodes*

R. A. ROHDE**

Extensive studies on respiration of free living nematodes have been made by Overgaard Nielsen (1949) and Santmeyer (1958). While Overgaard Nielsen included a few measurements on plant-parasitic forms, Santmeyer's data are confined to *Panagrellus redivivus* (Linn., 1767) Goodey, 1945, a particulate feeder. In both of these studies, $O_2$ uptake was measured in chambers where $CO_2$ had been quantitatively removed with KOH, under the assumption that the absence of $CO_2$ had no effect on the respiration rate. The following studies show that this is apparently not the case with certain plant-parasitic nematodes and that respiration rate is dependent on the amount of $CO_2$ present.

### MATERIALS AND METHODS

A Cartesian diver ultramicrorespirometer similar to the instrument used by Overgaard Nielsen (1949) was used in all studies. This instrument was selected for use because the numbers of nematodes available were limited and respiration measurements could be made with small numbers of animals.

The respirometer was constructed following the design of Holter and Linderstrom-Lang (1943), with only slight modifications. Divers were made of pyrex glass and had a total volume of 7-9 microliters. Diver necks were coated with silicone.

To load divers, the desired number of nematodes was drawn into a one microliter delivery pipette and the contents expelled into the diver bulb. A 0.5 microliter drop of 0.1 *N* KOH was suspended at the bottom of the diver neck, and a 0.5 microliter drop of paraffin oil was suspended midway up the neck. Sufficient flotation medium was placed in the mouth of the diver neck to allow the loaded diver to come to equilibrium near atmospheric pressure.

Studies on the influence of various concentrations of $CO_2$ were made using an application of the method of Pardee (1949). Pardee's solution, when charged with a given concentration of $CO_2$, will act as a "buffer" to maintain a given $CO_2$ pressure within an enclosed space. The physical properties of the solution are such that it may be used in place of KOH in the diver neck. The desired concentration of $CO_2$ was bubbled through the Pardee's solution, as well as the flotation medium of the vessel to be used for that concentration, for 12 hours before each experiment. All solutions were kept in the diver water bath during bubbling. The water bath was held to within 0.01°C of 25°C.

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Scientific Article No. *A848*, Contribution No. *3139* of the Maryland Agricultural Experiment Station, Department of Botany.

This research was supported in part by a grant from the Rockefeller Foundation.

**The author is indebted to Dr. E. J. Boell, Gibbs Memorial Laboratory, Yale University, New Haven, Conn. for his many helpful suggestions concerning the construction and use of the Cartesian Diver respirometer.

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TABLE 1.—Respiration rates of various nematodes 6-8 hours after extraction from soil. Each value was determined from duplicate divers containing 1-4 adult females. Ranges and mean values are given when more than one determination was made. Divers sealed with KOH, water bath at 25°C.

| Nematode species | No. of trials | Ave. wt., micrograms | Oxygen consumption/hr. microl./individual | Oxygen consumption/hr. l./kg. fresh wt. |
|---|---|---|---|---|
| *Trichodorus christiei* | 5 | 1.12 | 1.06 (.73-1.50) | .95 (.87-1.30) |
| *Tylenchorhynchus martini* | 1 | .90 | 3.31 | 3.72 |
| *Pratylenchus penetrans* | 5 | .14 | .42 (.30-.60) | 2.96 (2.11-4.22) |
| *Criconemoides* sp. | 1 | .30 | .68 | 2.27 |
| *Hemicycliophora* sp. | 3 | 1.60 | .69 (.64-.76) | .43 (.38-.54) |
| *Hoplolaimus tylenchiformis* | 1 | 2.00 | .40 | .20 |
| *Panagrellus redivivus* | 1 | 1.25 | 8.10 | 6.40 |

For all experiments, duplicate divers were used for each treatment and a control diver without nematodes was used for each treatment. Three levels of $CO_2$ were used for each experiment; air with $CO_2$ removed by means of KOH, normal air (0.3% $CO_2$), and air with either 1% or 2% $CO_2$ added.

Nematodes used were extracted from greenhouse pot cultures and the first respiration measurements were made within about 2 hours after extraction was started. Adult females were selected for uniform size, rinsed several times with sterile water, and stored in aerated tap water in the diver water bath. Rinse water was saved for use in control divers. Four adult females were used in each diver in most cases and weights of all nematodes used were determined from optical measurements using the method of Andrassy (1958).

## Results and Discussion

Preliminary respiration studies were made with several species of plant-parasitic nematodes as well as with *P. redivivus*. Results are given in Table 1. Measurements of extraction water and rinse water indicated that microorganisms were probably not a complicating factor. Respiration rates obtained were similar to those of Overgaard Nielsen (1949) and Santmeyer (1958). Santmeyer found that the respiration rate of *P. redivivus* dropped considerably during the initial 24 hours of starvation and then became more or less stable. However, Overgaard-Nielsen obtained essentially the same respiration rates making daily readings on nematodes starved in water over a 7 day period. Results with plant-parasitic species did not follow either of these patterns (Fig. 1). The respiration rate of *Pratylenchus penetrans* (Cobb, 1917) Filipjev and Steckhoven, 1941 decreased constantly over an 11 day period. After this time, death of individuals during runs made results erratic.

Long term experiments using the same population of nematodes for successive runs showed that the respiration rate of those nematodes in divers became lower than the rate of those nematodes kept in aerated water. Figure 1 shows a typical experiment with four separate rate determinations on the same population of nematodes spaced over a period of 11 days. The respiration rate at the beginning of each determination was higher than the rate at the end of the preceding one. Several series of experiments were run with plant parasites and the same effect was always noted. It was also observed that those nematodes in divers with a KOH seal became less active than those kept in aerated water.

Experiments using Pardee's solution in place of KOH were designed to explore the possibility that nematodes in divers with a KOH seal were respiring at a reduced rate because $CO_2$ was absent. Respiration rates of *P. penetrans* and *Hoplolaimus tylenchiformis* Daday, 1905 were found to be highest in air and lower in either higher concentrations of $CO_2$ or in air with $CO_2$ removed (Figs. 2 & 3). The same effect was noted whether nematodes were in water, pH 6.5 phosphate buffer, or pH 8 Trishydroxymethylamino Methane buffer. Evidently $CO_2$ is limiting only when its concentration drops below that of the atmosphere, and concentrations above this level inhibit respiration.

A single experiment run with freshly hatched larvae of *Meloidogyne hapla* Chitwood, 1949 gave similar results. In this case, too few individuals were used to give a measurable $O_2$ uptake in divers with no $CO_2$ or 1% $CO_2$, but the same number of individuals showed a high rate of respiration in divers containing air.

A stimulatory effect of low concentrations of $CO_2$ was observed by Searle and Reiner (1941) working with trypanosomes. The activation of organisms which had become motionless in $CO_2$-free medium and the increase of $O_2$ uptake and glucose utilization were explained on the basis of $CO_2$ fixation.

The inhibitory effect of increased concentrations of $CO_2$ on plant parasitic nematodes has been observed by several workers. Hastings and Newton (1937) found that either $CO_2$ or rotten bulb infusion prevented dormant specimens of *Ditylenchus dipsaci* (Kuhn, 1857) Filipjev, 1936 from recovering motility. Gillard, D'Herde and Van den Brande (1958) have shown that

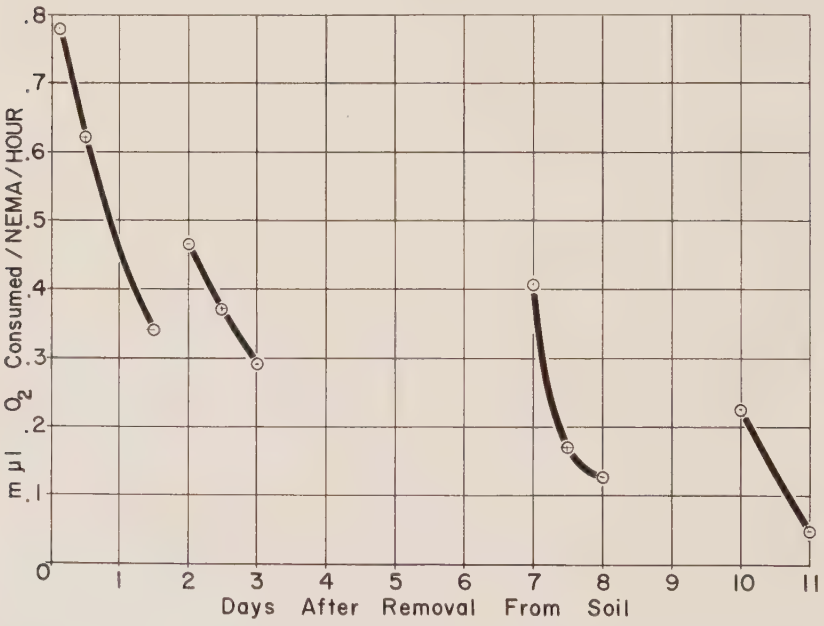


Figure 1. Respiration of *Pratylenchus penetrans* determined in divers containing KOH as a $CO^2$ absorbent. Separate curves were determined from duplicate samples of 4 adult females taken from a population stored in aerated water. It can be seen that the decrease in respiration rate of individual samples is much more rapid than decrease in respiration rate for the population as a whole. Nematodes were stored and respiration measurements were made at 25°C.

$CO_2$ inhibits larval emergence from cysts of *Heterodera rostochiensis* Wollenweber, 1923.

An interesting possibility arises when one considers that $CO_2$ released by respiring roots may decrease the metabolic activity of nematodes in the root area. Kühn (1959) has proposed that an orthokinetic substance, as yet unknown, decreases activity of nematodes in the proximity of roots and is responsible for nematodes' staying in the root area. It is possible that high concentrations of $CO_2$ which build up in the vicinity of respiring roots have this effect, although a great deal more evidence than that presented above is necessary to prove this theory.

## CONCLUSIONS

1. Suffcient sensitivity is possible with the Cartesian diver ultra-microrespirometer to permit respiration measurements on small numbers of plant-parasitic nematodes.

2. Respiration rates of plant-parasitic nematodes decrease constantly from the time of removal from the host plant until death occurs.

3. Respiration rates of certain plant-parasitic nematodes are higher in air than in vessels without $CO_2$ or with $CO_2$ levels higher than that of air.

4. A hypothesis is presented that $CO_2$ released from respiring roots acts as an orthokinetical stimulus to decrease activity and prevent nematodes from leaving the root area.

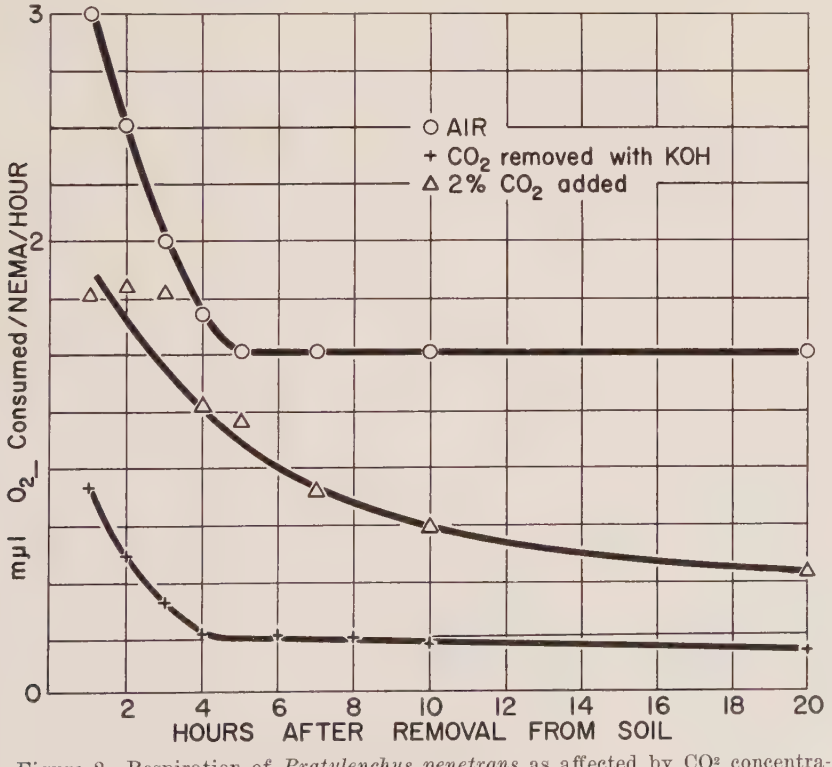


Figure 2. Respiration of *Pratylenchus penetrans* as affected by $CO_2$ concentration. Each curve was determined from duplicate divers containing 4 adult females each, and all measurements were made at 25°C.

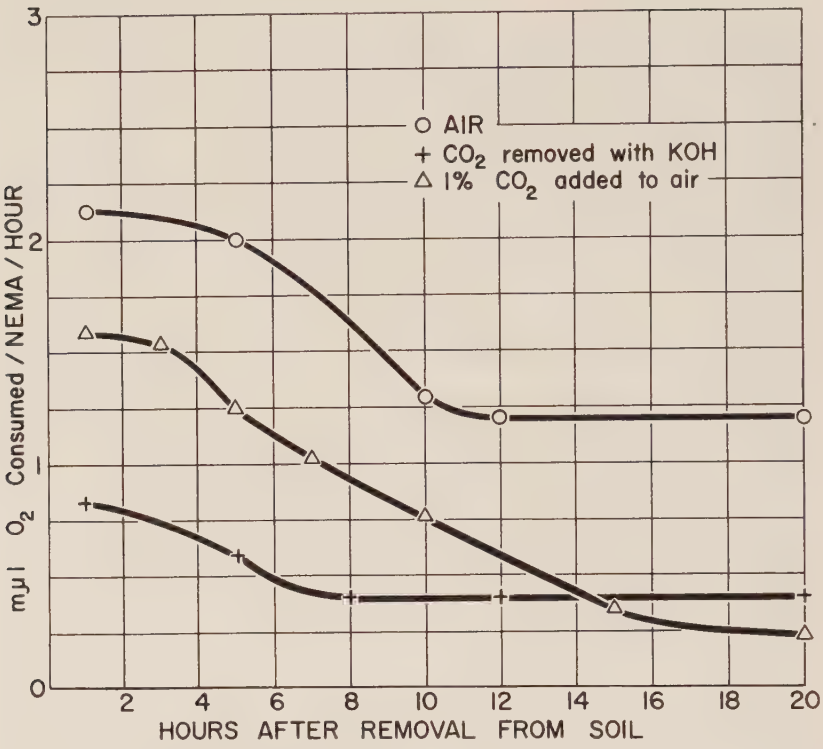


Figure 3. Respiration of *Hoplolaimus tylenchiformis* as affected by $CO^2$ concentration. Each curve was determined from duplicate divers containing 4 adult females each and all measurements were made at 25°C.

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## Additional Hemiurid Trematodes from Hawaiian Fishes*

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This paper is based on specimens collected by Dr. Hilda L. Ching during August, 1959, at the Waikiki Aquarium and at the Coconut Island Marine Laboratory of the University of Hawaii. Host identifications were confirmed by Dr. William A. Gosline. The authors recently reported 7 species of hemiurids from Hawaii (Manter & Pritchard, 1960). The present collection includes 10 additional species, 6 of which are new; the other 4 constitute new records for Hawaii.

All egg measurements are in microns and all other measurements are in millimeters.

### SUBFAMILY APHANURINAE SKRJABIN and GUSCHANSKAJA, 1954
(Synonym: Ahemiurinae Chauhan, 1954)

The Aphanurinae are characterized by cuticular plications, no ecsoma, and one or two compact vitellaria. Genera: *Aphanurus* Lühe, 1901; *Ahemiurus* Chauhan, 1954; *Myosaccium* Montgomery, 1957; and the new genus named herein. We follow Yamaguti (1958) in considering *Chauhanurus* Skrj. & Gusch., 1954 as a synonym of *Aphanurus*.

### *Duosphincter zancli*, n. gen., n. sp. (Figs. 1-2)

HOST: *Zanclus canescens* (L.), kihikihi or moorish idol (Zanclidae); 27 specimens from 8 of 23 hosts.

LOCATION: Stomach

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll., No. 39167.

DESCRIPTION (based on 27 specimens): Body, without ecsoma, 0.871 to 1.354 long by 0.201 to 0.281 wide (width about same along entire length), anterior end rounded, posterior end slightly more pointed; both forebody and hindbody bent dorsally from acetabulum. Cuticula thin, horizontal plications encircle body along its entire length. Preoral lobe small. Oral sucker rounded, 0.141 to 0.181 wide by 0.101 to 0.168 long; mouth ventral, pyriform with pointed anterior end, surrounded by well-developed sphincter. Acetabulum ⅓ body length from anterior end, somewhat protuberant, 0.160 to 0.181 wide by 0.121 to 0.176 long; aperture triangular with one point directed posteriorly, also surrounded by well-developed sphincter. Sucker ratio 1:1 to 1.2. Pharynx 0.032 to 0.050 long by 0.058 to 0.069 wide; esophagus short, 0.019 to 0.024 in diameter; ceca slender at origin, then widening and extending to near posterior end of body.

Testes diagonal with either right or left testis anterior, rounded, 0.070 to 0.120 long by 0.096 to 0.120 wide, contiguous or close together. Seminal vesicle tubular but not slender, winding (4 or 5 turns) dorsally between mid-acetabulum and level of genital pore. Pars prostatica narrow and straight extending ventrally or posteroventrally into sinus sac; prostatic cells surround pars prostatica and terminal part of seminal vesicle while smaller gland cells surround both pars prostatica and metraterm as they enter sinus sac (Fig. 2); pars prostatica joins metraterm at base of sinus sac to form short hermaphroditic duct. Sinus sac small, tubular, enclosing hermaphroditic duct, partially protrusible. Genital pore median or slightly submedian, at level of bifurcation or immediately posterior to bifurcation.

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*Studies from the Department of Zoology, University of Nebraska, No. 323. This study was supported by a grant from the National Science Foundation.

Ovary to left, rounded, 0.048 to 0.120 long by 0.096 to 0.160 wide, overlapped by posterior testis and anterior vitellarium. Vitellaria compact, diagonal, overlapping, 0.072 to 0.128 long by 0.086 to 0.138 wide, anterior vitellarium usually to left and usually wider. Uterus fills postacetabular spaces posterior, dorsal, and anterior to gonads. A small sphincter just outside sinus sac marks beginning of very short metraterm; metraterm not especially muscular, enters sinus sacs and joins pars prostatica. Eggs yellowish, 21 to 26 by 13 to 17.

Excretory pore subterminal, ventral; a small, papilla-like structure protrudes in 7 specimens. Excretory vesicle not fully traced, but division occurs in region of gonads and crura extend forward and unite dorsal to pharynx.

GENERIC DIAGNOSIS OF *Duosphincter*: Hemiuridae, Aphanurinae: Body small, without ecsoma; horizontal cuticular plications encircle body along its entire length; oral sucker subterminal, mouth surrounded by well-developed sphincter; ceca extending to near posterior end of body; acetabulum preequatorial, protuberant, aperture with well-developed sphincter; genital pore ventral to bifurcation; testes diagonal, close together, postacetabular in middle ⅓ of body; seminal vesicle tubular, winding in forebody, rarely reaching posterior to mid-acetabulum; pars prostatica short, surrounded by prostatic cells; sinus sac short, tubular, enclosing hermaphroditic duct; ovary ovoid, posttesticular, vitellaria in two ovoid masses, postovarian, diagonal to tandem; uterus fills postacetabular spaces posterior, dorsal, and anterior to gonads; metraterm short, not especially muscular but separated from uterus by small sphincter; excretory pore subterminal, ventral; excretory vesicle Y-shaped, crura joining anteriorly.

The name *Duosphincter* is from *duo* (= two) and *sphincter* (= a closing muscle) referring to the well-developed sphincter muscles present in both the oral sucker and the acetabulum.

DISCUSSION: The tubular, winding shape and the preacetabular position of the seminal vesicle are peculiar to *Duosphincter*. In *Aphanurus* and *Ahemiurus* the seminal vesicle is saccular and entirely postacetabular; in *Myosaccium* it is saccular and most of it is immediately postacetabular, although it extends dorsal to the acetabulum. The sphincter muscles of the oral sucker and acetabulum are absent in the other genera. *Duosphincter* differs from *Aphanurus* in possessing two vitelline glands, a short preacetabular pars prostatica, a shorter and less muscular hermaphroditic duct, a more posterior genital pore, and plications that extend horizontally around the body rather than at an angle. It differs from *Ahemiurus* in having a short preacetabular pars prostatica, a shorter and less muscular hermaphroditic duct, and diagonal vitellaria. It differs from *Myosaccium* in the complete absence of a prostatic vesicle, the shorter sinus sac, the lack of an ejaculatory duct, larger gonads, cuticular plications over all the body, and the non-filamented eggs.

### SUBFAMILY DINURINAE LOOSS, 1907

The Dinurinae are characterized by cuticular plications, an ecsoma, and long, winding vitellaria. Genera: *Dinurus* Looss, 1907; *Ectenurus* Looss,

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All figures drawn with the aid of a camera lucida. The projected scale has its value indicated in millimeters. Abbreviations used: *ce*, cecum; *ex*, excretory system; *gp*, genital pore; *hd*, hermaphroditic duct; *mt*, metraterm; *pa*, preacetabular pit; *pp*, pars prostatica; *prv*, prostatic vesicle; *sph*, sphincter; *ss*, sinus sac; *sv*, seminal vesicle; *ut*, uterus; *uts*, uterine swelling; *vt*, vitellaria.

PLATE I

Fig. 1. *Duosphincter zancli* from *Zanclus canescens*, holotype; ventral view.

Fig. 2. *D. zancli* from *Z. canescens*, paratype; lateral view of terminal genital ducts.

Fig. 3. *Ectenurus lepidus* Looss, 1907, from *Decapterus pinnulatus;* ventral view.

Fig. 4. *Lecithocladium chingi* from *Naso* sp., holotype; ventral view.

Fig. 5. *L. chingi* from *Naso* sp., paratype; ventral view of terminal genital ducts (a posterior portion is displaced to the left; its actual position is dorsal to the pars prostatica shown on the right).

Fig. 6. *L. chingi* from *Naso* sp., paratype; ventral view of ovary and vitellaria.

Fig. 7. *L. chingi* from *Naso* sp., paratype; ventral view of excretory vesicle in ecsoma.

1907; *Lecithocladium* Lühe, 1901 (syn. *Clupenurus* Srivastava, 1935); *Magnacetabulum* Yam., 1934.

Manter (1947) named *Parectenurus,* differentiating it from *Ectenurus* because the seminal vesicle was not tripartite even though the paratype specimen from *Synodus foetens* was figured with an indistinctly tripartite seminal vesicle. The difference is largely one of degree, and we now regard *Parectenurus* a synonym of *Ectenurus.*

Yamaguti (1954, 1958) considered *Parectenurus* as a synonym of *Magnacetabulum* Yam., 1934 although *Parectenurus* has a well-developed sinus sac and *Magnacetabulum* has none. In 1953, Yamaguti described *M. leiognathi* which has a "ductus hermaphroditicus . . . enclosed in a very thin membranous capsule" (i.e. sinus sac). Since *Magnacetabulum* is entirely without a sinus sac, *M. leiognathi* should be transferred to *Ectenurus.*

The new combinations are: *Ectenurus americanus* (Manter, 1947) [synonyms: *Parectenurus americanus* Manter, 1947; *Magnacetabulum americanum* (Manter, 1947) Yam., 1954] and *Ectenurus leiognathi* (Yam., 1953) [synonym: *Magnacetabulum leiognathi* Yam., 1953].

*Ectenurus lepidus* Looss, 1907 (Fig. 3)

HOSTS (both new records): *Decapterus pinnulatus* (Eydoux & Souleyet), 'opelu, mackerel scad, or 'opelu-mama (Carangidae); 5 specimens from 3 hosts. *Anampses cuvieri* Quoy & Gaimard, 'opule or hilu (Labridae, wrasses); 1 from 1 host.

LOCATION: Intestine

SPECIMEN deposited: U. S. Nat. Mus. Helminth. Coll., No. 39176.

DISCUSSION: *E. lepidus* has been reported principally from carangids in the Adriatic, Mediterranean, and Black seas, and at Aberdeen, Scotland, Wellington, New Zealand, and now Hawaii. Vlassenko (1931) found no anterodorsal papillae on his specimen from the Black Sea; Manter (1954) observed that the papillae "are inconspicuous and not always evident"; very small ones were observed on only one of the Hawaiian specimens, and that specimen was flattened with the preoral lobe bent ventrally over the oral sucker.

In most respects the Hawaiian specimens agree with specimens of *E. lepidus* reported from other localities. The sucker ratio is both smaller and larger than reported by Looss (1:2.4 to 2.8 as compared with 1:2.5) and it reaches the lower limit reported by Manter (1:2.8 to 3). The eggs average smaller (16 by 10) than those reported by Looss (20 by 10), but the range (13 to 19 by 8 to 11) overlaps the limits reported by Manter (18 to 21 by 9 to 10). Like the New Zealand specimens, the sinus sac is elongate but does not reach the acetabulum.

*Lecithocladium chingi,* n. sp. (Figs. 4-7)

HOST: *Acanthurus mata* (Cuvier), (Acanthuridae, surgeonfishes); 2 specimens from 1 of 3 hosts.

*Naso ?brevirostris* (Cuvier & Valenciennes) or *N. ?unicornis* (Forskål), kala or unicorn fish (Acanthuridae, surgeonfishes—the two species are so similar that all differentiations are uncertain); 21 specimens from 10 of 28 hosts. Type host.

*Melichthys vidua* (Solander), humuhumu-hi'u-kole or humuhumu-uli (Balistidae, triggerfishes); 2 specimens from 1 of 5 hosts.

LOCATION: Stomach

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll., No. 39168.

DESCRIPTION (based principally upon 10 mature specimens and 5 young specimens): Body filiform, with ecsoma; body proper 2.144 to 3.564; ecsoma 0.436 to 2.345 long, retracted no more than 0.4 and usually not at all; total length 3.150 to 5.494; width 0.503 to 0.992 at posterior end of body. Cuticular plications encircle body, sometimes less distinct posteriorly. Oral sucker 0.241 to 0.369 wide by 0.221 to 0.315 long, surmounted by short preoral lobe. Acetabulum $^1/_5$ to $^1/_3$ body length from anterior end, 0.281 to 0.375 wide by 0.255 to 0.375 long. Sucker ratio 1:1.0 to 1.3. Pharynx 0.168 to 0.288 long by 0.134 to 0.201 wide, slightly narrowed anteriorly; esophagus first rounded 0.087 to 0.107 in diameter, then directed anterodorsal or dorsal for short distance; ceca, often containing blood, arise dorsally, enlarge and extend dorsolaterally along posterior half of pharynx then turn posteriorly, tips reach almost to posterior end of ecsoma.

Testes well separated from acetabulum, rounded, almost symmetrical or somewhat diagonal, similar in size, 0.101 to 0.255 long by 0.141 to 0.235 wide. Seminal vesicle partially or wholly anterior to testes, thick walled, saccular but usually constricted near middle to give almost bipartite appearance, 0.215 to 0.369 long by 0.109 to 0.154 wide; short, narrow duct leads posteriorly from anterior end of seminal vesicle to join pars prostatica. Pars prostatica very long, extends posteriorly almost to ovary, turns anteriorly and follows a sinuous course between testes and ventral to seminal vesicle, dorsal to acetabulum, and joins sinus sac near anterior edge of acetabulum; prostatic cells large and numerous, decreasing slightly in size and number near sinus sac. Sinus sac curves slightly to genital pore, 0.436 to 0.616 long by 0.034 to 0.088 wide. Hermaphroditic duct well developed with muscular wall, free in sinus sac, slightly protruded from genital pore in some specimens; protruded tip slender and smooth followed by short, expanded portion seeming to bear minute papillae. Genital pore median, ventral to anterior half of oral sucker in all but two specimens, ventral to posterior half of oral sucker in latter.

Ovary 0.067 to 0.201 long by 0.121 to 0.255 wide, slightly reniform, ventral and more or less median. Vitellaria consisting of paired symmetrical principal masses immediately postovarian, 0.067 to 0.121 in diameter, one with 3 lobes, other with 4 lobes; lobes very long and very coiled, encircling ovary. Initial part of uterus contains sperm cells; uterine coils descend on left, enter ecsoma a litle to as much as $^1/_3$ its length, then ascend on right side dorsal to gonads and loop 2 or 3 times before following anterior part of pars prostatica to sinus sac; uterus joins pars prostatica at base of sinus sac. Eggs numerous, yellowish, 14 to 18 by 8 to 11.

Excretory pore terminal; in the ecsoma, the median stem of excretory vesicle ascends between ceca with repeated forward and backward turnings (Fig. 7); anterior to ecsoma it becomes more straight, dividing at level of gonads; crura diverge and continue ventrally lateral to gonads and acetabulum and forward beside oral sucker, turn backward, narrow greatly, and parallel themselves back to level of gonads where they subdivide; one branch continues almost to posterior end of body proper.

Ten immature specimens (rudimentary gonads and no eggs) had a total length of 1.474 to 3.792 and were widest at acetabular level.

COMPARISONS: The length of the pars prostatica (almost four times the length of the seminal vesicle) and its posterior extent (posterior to the seminal vesicle and almost to the ovary) distinguish *L. chingi* from all other species of *Lecithocladium*. The vitelline lobes are unusually long and sinuous,

and they extend from a pair of well developed vitelline masses. The alternately anterior and posterior course of the excretory vesicle in the ecsoma is undescribed and is not figured for any other species, nor is the narrow, posterior extension of the lateral vessels.

*L. chingi* seems most similar to *L. magnacetabulum* Yam., 1934 (syn. *L. pagrosomi* Yam., 1934). The body size, egg size, location of the ecsoma invagination, and proximity of seminal vesicle to acetabulum seem closer to the description of *L. pagrosomi.* The sucker ratio of *L. chingi* (1:1.0-1.3) may be smaller, but it may overlap the ratio for *L. magnacetabulum* (1:1.2-1.55). The pars prostatica of *L. magnacetabulum* may loop backward but only partially overlaps the seminal vesicle, and its total length is not longer than the seminal vesicle.

Yamaguti (1958:280) questioned the identification of *L. magnacetabulum* from New Zealand by Manter (1954), pointing out that an egg-size range of 19 to 30 by 9 to 15 is too great for a single species. Three specimens of the New Zealand material were available for restudy. The size range reported is, in fact, misleading. The normal range, or at least the range of eggs selected as typical or normal, seems to be 20 to 26 by 11 to 14. There was slightly greater variation, but the smaller eggs were clearly abnormal with extra thick shells and eggs of 26 to 27 microns had thinner shells. These three specimens were clearly a single species. This correction does not help to identify the species since the egg size is exactly between that reported for *L. magnacetabulum* and *L. pagrosomi.* The size of the acetabulum of the New Zealand specimens is more like that of *L. pagrosomi.* In view of the tendency toward instability of egg size, we still believe *L. pagrosomi* should be considered a synonym of *L. magnacetabulum.*

### SUBFAMILY LECITHASTERINAE ODHNER, 1905
### (syn. Derogenetinae Odhner, 1921)

Hemiurids without ecsoma; without cuticular plications; with vitellaria unlobed, lobed, digitate, or with seven separated parts.

### *Aponurus acanthuri,* n. sp. (Fig. 8)

HOST: *Acanthurus sandvicensis* (Streets), manini or convict tang (Acanthuridae, surgeonfishes); 1 specimen from 56 hosts.

LOCATION: Stomach

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll., No. 39170.

DESCRIPTION: Body elongated, more or less cylindrical, smooth, without ecsoma, 1.822 long by 0.322 wide, widest immediately posterior to acetabulum and posterior to ovary; both ends rounded. Oral sucker 0.154 wide by 0.134 long; mouth ventral, surmounted by preoral lobe. Acetabulum in second 1/5

PLATE II

Fig. 8. *Aponurus acanthuri* from *Acanthurus sandvicensis,* holotype; ventral view.

Fig. 9. *Genolinea lobata* from *Acanthurus sandvicensis,* holotype; ventral view.

Fig. 10. a-c. *G. lobata* from *A. sandvicensis,* 3 paratypes; views of ovary with vitellaria.

Fig. 11. *Lecithochirium microstomum* Chandler, 1935, from *Dascyllus albisella;* ventrolateral view.

Fig. 12. *L. microstomum* from *D. albisella;* ventral view with ecsoma retracted.

Fig. 13. *L. microstomum* from *Chaetodon auriga;* ventral view with ecsoma extended.

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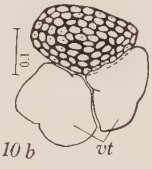


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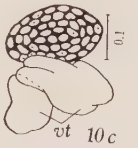


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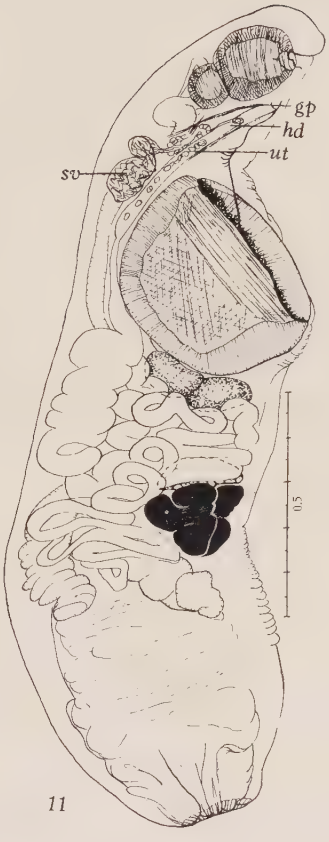


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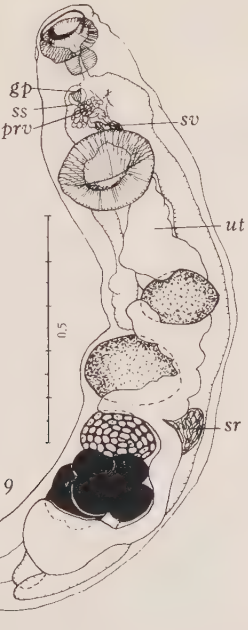


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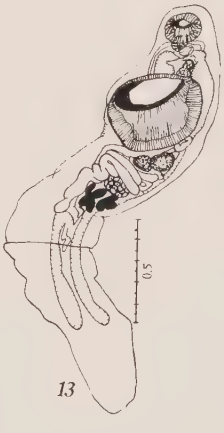


13

of body length, 0.235 wide by 0.214 long, aperture rounded. Sucker ratio 1:1.5. Pharynx 0.060 long by 0.087 wide; esophagus short; ceca relatively wide with irregular margins, extending to near posterior end of body where tips turn anteroventrally and end blindly.

Testes separated by uterus, diagonal, rounded, 0.096 to 0.107 long by 0.115 to 0.120 wide, near midbody, anterior testis 0.255 posterior to acetabulum. Seminal vesicle preacetabular, saccular, small, about 0.080 long by 0.048 wide. Pars prostatica swollen and vesicular, small, about 0.040 in diameter, surrounded by prostatic cells; pars prostatica joins uterus at base of sinus sac. Sinus sac globular containing only the hermaphroditic duct; genital atrim lacking. Genital pore at level of intestinal bifurcation, quite probably median.

Ovary posttesticular, median, ovoid, 0.114 long by 0.155 wide. Seminal receptacle elongated but smaller than ovary, to left of ovary. Vitellaria composed of seven rounded to oval bodies 0.048 to 0.064 long by 0.048 to 0.080 wide, arranged in two horizontal rows (4 anterior and 3 posterior) *or,* differently interpreted, two contiguous groups of 4 and 3 (4 right and 3 left); anterior vitellaria contiguous with ovary. Uterus narrows dorsal to acetabulum but no metraterm differentiated. Mature, uncollapsed eggs measure 21 to 24 by 12 to 14, but collapsed eggs measure 29 to 32 by 16 to 19. Excretory pore terminal; anterior extent of vesicle not determined but, judging from the distribution of refractive granules, the crura joins dorsal to the oral sucker.

DISCUSSION: This species is more elongate than other species in the genus. It is distinctive in that uterine coils occur between the testes and also between the testes and ovary so that no two of these organs are contiguous. This condition occurs to some degree in *A. synagris* Yam., 1953 which differs in that the ceca do not extend posterior to the uterus, the vitellaria are not horizontally arranged, the testes are more symmetrical, and the eggs are somewhat larger.

*Genolinea lobata,* n. sp. (Figs. 9-10)

HOST: *Acanthurus sandvicensis* (Streets), manini or convict tang (Acanthuridae, surgeonfishes); 4 specimens from 3 of 56 hosts.

LOCATION: Stomach

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll., No. 39169.

DESCRIPTION (based on 4 specimens): Body small, without ecsoma, 1.434 to 2.352 long by 0.268 to 0.348 wide at acetabulum and most of hindbody, forebody slightly tapered, both ends rounded. Oral sucker 0.115 to 0.144 wide by 0.088 to 0.121 long, embedded in body with a rim of body wall around mouth, rim thicker dorsally. Acetabulum about ¼ from anterior end, rounded, 0.192 to 0.235 wide by 0.173 to 0.221 long, aperture horizontal. Sucker ratio 1:1.6 to 1.7. Pharynx 0.051 to 0.074 long by 0.064 to 0.080 wide; esophagus narrow and short; ceca widen immediately at bifurcation, turn and extend to near posterior end of body, end blindly.

Testes diagonal, separated from acetabulum and from each other by uterus, rounded to oval, 0.112 to 0.168 long by 0.136 to 0.203 wide; seminal vesicle tubular, slender, coiled once or twice, preacetabular; pars prostatica expanded into small, rounded prostatic vesicle, surrounded by numerous, small prostatic cells; sinus sac short, containing muscular hermaphorditic duct; genital pore median to submedian at level of intestinal bifurcation.

Ovary oval, 0.112 to 0.198 long by 0.147 to 0.205 wide; seminal receptacle

elongate oval, dorsal and partly anterior to ovary, 0.201 to 0.248 long by 0.136 to 0.160 wide; vitellaria two, immediately postovarian, diagonal to symmetrical, contiguous, variously lobed (Figs. 9-10), 0.128 to 0.200 long by 0.120 to 0.203 wide; uterus descends almost to tips of ceca (but not beyond) and ascends dorsally, separating gonads and expanding between anterior testis and acetabulum, narrowing dorsal to acetabulum and joining pars prostatica at base of sinus sac. Eggs 27 to 32 by 14 to 18.

Excretory pore terminal, excrttory crura extend forward to oral sucker, turn somewhat backwards and medianly, and seem to terminate dorsal to pharynx without joining.

*G. lobata* is named for the lobed condition of the vitellaria.

DISCUSSION: This species has all the generic characteristics of *Genolinea* except the "unlobed, tandem or slightly diagonal vitellaria" and the branches of the excretory system uniting dorsal to the pharynx. It seems to us that a new genus would be unjustified, and for the present the species is added to *Genolinea* in which it is the only species with lobed vitellaria.

*G. lobata* resembles *G. manteri* Lloyd, 1938 but differs in smaller sucker ratio, smaller eggs, more anterior seminal vesicle, and the embedded oral sucker. In the latter respect *G. lobata* resembles *G. ampladena* Manter & Pritchard, 1960 (also from Hawaii), but the prostatic vesicle is less well developed, the sucker ratio is smaller, and the eggs are shorter.

*Hysterolecitha tinkeri* Manter & Pritchard, 1960

HOST: *Chaetodon fremblii* Bennett (Chaetodontidae, butterfly fishes); 2 specimens from 1 of 17 hosts. New host record.

LOCATION: Intestine

*Lecithaster stellatus* Looss, 1907

Synonym: *Lecithaster sayori* Yamaguti, 1938

HOSTS (both new records): *Acanthurus olivaceus* (Bloch and Schneider), na'ena'e (Acanthuridae, surgeonfishes); 1 specimen from 1 of 15 hosts.
*A. sandvicensis* (Streets), manini or convict tang (Acanthuridae, surgeonfishes); 3 specimens from 2 of 56 hosts.

LOCATION: Stomach and intestine

SPECIMEN DEPOSITED: U. S. Nat. Mus. Helminth. Coll., No. 39175.

BRIEF DESCRIPTION of Hawaiian specimens: 1.246 to 1.628 long by 0.375 wide (0.436 to 0.623 deep); oral sucker 0.114 wide by 0.094 to 0.141 long; acetabulum 0.241 wide by 0.201 to 0.275 long; sucker ratio 1:2.1; pharynx 0.063 to 0.080 long by 0.070 wide; seminal vesicle 0.121 to 0.201 long by 0.091 to 0.147 wide; eggs 13 to 16 by 8 to 11.

DISCUSSION: These specimens agree with the descriptions of *L. stellatus* as given by Looss (1907) and Yamaguti (1934, 1953). The pars prostatica is less S-shaped in the Hawaiian specimens.

Yamaguti (1938) described *Lecithaster sayori* from the large intestine of *Hyporhampus sajori* (Temm. & Schleg.) in Lake Hamana, acknowledging that "except for measurements this species is hardly distinguishable from *Lecithaster stellatus* Looss, 1907, but I would regard it as distinct in view of its habitat." The larger size of the Hawaiian specimens brings the measurements of *L. stellatus* even closer to those of *L. sayori*, and the wide variety of hosts reported for *L. stellatus* reduces the relative importance of "habitat." We consider *L. sayori* a synonym of *L. stellatus*.

SUBFAMILY LECITHOCHIRIINAE LÜHE, 1901

Hemiurids with ecsoma, non-plicated cuticula, and compact or digitate to winding vitellaria. Manter and Pritchard (1960) listed 17 genera in this subfamily. Four others should have been included: *Anahemiurus* Manter, 1947; *Erilepturus* Woolcock, 1935 (Syn. *Uterovesiculurus* Skrj. & Gusch., 1954); *Mecoderus* Manter, 1940; and *Tubulovesicula* Yam., 1934 (Syn. *Lecithurus* Pigulewsky, 1938).

The above concept of the Lecithochiriinae does bring together two more or less distinct groups of genera: those resembling the Dinurinae in having a postacetabular seminal vesicle and long winding vitellaria; and those more like *Sterrhurus* in having the seminal vesicle preacetabular or dorsal to the acetabulum and shorter vitellaria.

Yamaguti does not consider the cuticular plications as a generic character. He named (1934) three species which, although smooth-bodied, he placed in *Ectenurus* (*E. hamati, E. paralichthydis, E. platycephali*). We believe these belong in the genus *Erilepturus* Woolcock, 1935 as already indicated for the first two by Manter (1947). All three of these species showed a uterine swelling immediately before the union with the pars prostatica, although it was inconspicuous in *E. platycephali.* Skrjabin and Guschanskaja (1954) recognized the genus *Erilepturus* but named *Uterovesiculurus* for the three species with the uterine swelling. As will be noted below, we found this character to be of inconstant appearance within a single species (*Tubulovesicula angusticauda*). *Uterovesiculurus* is therefore considered a synonym of *Erilepturus. Erilepturus platycephali* (Yam., 1934) is a new combination.

*Lecithochirium magnaporum* Manter, 1940

HOST: *Dactyloptena orientalis* (Cuvier & Valenciennes), lolo-'oau (Dactylopteridae); 6 specimens from 1 of 3 hosts. New host and distribution record.

LOCATION: Stomach

SPECIMEN DEPOSITED: U. S. Nat. Mus. Helminth. Coll., No. 39174.

BRIEF DESCRIPTION of Hawaiian specimens: Body 2.111 to 3.136 long (including ecsoma extended none to 0.503 beyond body proper) by 0.630 to 0.670 wide; oral sucker 0.177 to 0.201 wide; acetabulum 0.409 to 0.469 wide; sucker ratio 1:2 to 2.4; preacetabular pit large, round, deep, and glandular; genital pore large, transverse, ventral to anterior part of pharynx with moderately conspicuous radiating muscles; seminal vesicle tripartite (posterior parts only slightly divided); sinus sac 0.096 to 0.128 long by 0.120 to 0.147 wide, containing tranversely oval, rounded, or pyriform prostatic vesicle and short, broad, muscular hermaphroditic duct with longitudinally striated wall; thin-walled, transparent cells fill available space within sinus sac; eggs 21 to 24 by 14 to 16 (collapsed eggs 19 to 24 by 10 to 14).

DISCUSSION: Montgomery (1957) reported *L. magnaporum* from La Jolla, California, adding that the specimens were larger (2 to 4.18 by 0.55 to 0.69) and had a larger sucker ratio (1:2.3 to 2.4).

The Hawaiian material was compared with 21 paratypes of *L. magnaporum* from the Galapagos Islands and differs principally in size, the somewhat less conspicuous radiations of the genital pore, the striated hermaphroditic duct, and the larger eggs; but each difference except body size is matched in one or more of the paratypes. The Hawaiian specimens are like the La Jolla specimens in size, but both collections are otherwise so similar to *L. magnaporum* from the Galapagos Islands that they are considered the same species.

*L. australis* Manter, 1954 is a very closely related species which differs in that the sinus sac is longer than broad and the genital pore is small, rounded, and without easily observed radiating muscles.

*Lecithochirium microstomum* Chandler, 1935 (Figs. 11-13)

HOSTS (all new host records): *Dascyllus albisella* Gill (Pomacentridae, damselfishes); 46 specimens from 5 of 20 hosts.

*Chaetodon auriga* Forskål (Chaetodontidae, butterfly fishes); 5 specimens from 1 of 28 hosts.

*C. corallicola* Snyder (Chaetodontidae, butterfly fishes); 2 specimens from 1 of 8 hosts.

*C. miliaris* Quoy & Gaimard (Chaetodontidae, butterfly fishes); 3 specimens from 2 of 43 hosts.

*Hemitaurichthys zoster* (Bennett), (Chaetodontidae, butterfly fishes); 1 specimen from 1 host.

*Bodianus bilunulatus* (Lacépède), 'a'awa (Labridae, wrasses); 1 specimen from 1 of 7 hosts.

LOCATION: Stomach; stomach and intestine; intestine.

SPECIMENS DEPOSITED: U. S. Nat. Mus. Helminth. Coll., No. 39173.

DISCUSSION: Published descriptions of *L. microstomum* based on collections from various hosts in the Gulf of Mexico and the tropical American Pacific have increased the size range by decreasing the lower limits, i.e. body 1.5* to 4.8 long by 0.4* to 1.0 wide, oral sucker 0.125* to 0.200 wide, acetabulum 0.363* to 0.540 wide, sucker ratio 1:2.3 to 2.9*, eggs 16 to 24 by 10 to 13. Testes are as large or larger than the oral sucker, more or less diagonal, and the anterior testis may be contiguous with the acetabulum or separated from it by one or two loops of the uterus. We believe (Manter & Pritchard, 1960) the species is characterized by a non-glandular or inconspicuously glandular preacetabular pit, vitelline lobes as long as wide or up to twice as long, a cylindrical to pyriform sinus sac containing a spherical prostatic vesicle and muscular hermaphroditic duct. The *L. microstomum* reported from *Pseudupeneus multifasciatus* in Hawaii falls within the above limits and agrees with the specific diagnosis.

These 58 specimens, like those from the Galapagos Islands, tend to be smaller (0.831 to 2.781 long by 0.281 to 0.838 wide) and have a smaller sucker ratio (usually 1:2.3 or 2.4 but two specimens each are 1:2 and 1:2.5). The smallest specimens are probably progenetic. The sinus sac seems to be the more "open" type with some muscle strands converging posterior to the prostatic vesicle and some strands continuing posteriorly into the parenchyma. The most conspicuous variation, however, is the consistently smaller size of the testes (smaller than the oral sucker), their immediately postacetabular location, and their symmetrical, or practically symmetrical, relationship. We have reviewed more than 40 specimens of *L. microstomum* from the Galapagos Islands and Tortugas, Florida, and can confirm that all possess an easily observed rounded to pyriform sinus sac, and the large majority have large, more or less diagonal testes.

Of nine specimens from *Euthynnus alletterata* at the Galapagos Islands, two have almost symmetrical testes, and three have testes slightly smaller than the oral sucker while two others have testes about equal in size to the

*This measurement indicated by scale accompanying Sogandares-Bernal and Hutton's (1959) figure.

oral sucker. Because of these intergradations, we feel at present that the differences are subspecific.

*Lecithochirium spiravesiculatatum*, n. sp. (Figs. 14-15)

Host: *Gymnothorax undulatus* (Lacépède), puhi-laumilo (Muraenidae, moray eels); 1 from 1 host.

Location: Found in dish containing both stomach and intestine.

Holotype: U. S. Nat. Mus. Helminth. Coll., No. 39172.

Description: Body elongate, with retracted ecsoma, 3.303 long by 0.858 wide at level of acetabulum; both anterior and posterior ends slightly tapered and bluntly rounded. Preacetabular pit median, immediately anterior to acetabulum, non-glandular with transverese aperture. Forebody muscular, parenchyma vesicular. Oral sucker subterminal, rounded, 0.335 wide by 0.322 long. Acetabulum about ⅓ body length from anterior end, rounded, 0.583 wide by 0.603 long, aperture longitudinally elongate. Sucker ratio 1:1.7. Pharynx small, rounded, 0.101 long by 0.114 wide; esophagus inflated, thin-walled, 0.144 long by 0.160 wide; ceca inflated in forebody but muscular and contracted in hindbody, ending blindly anterior to base of ecsoma.

Testes symmetrical, immediately postacetabular, small, 0.101 long by 0.107 to 0.134 wide. Seminal vesicle tripartite, conspicuous constrictions separating parts; posterior part thin-walled, 0.281 long by 0.067 wide, lying obliquely along right anterior edge of acetabulum; middle part 0.194 long by 0.074 wide with thin muscular wall 0.010 thick, lying anterodextrad to posterior part; anterior part directed medianly, 0.087 long by 0.054 wide with muscular wall 0.020 thick. Seminal vesicle joined to pars prostatica by thin-walled duct 0.053 long. Prostatic cells well developed; pars prostatica short. Sinus sac pyriform, very muscular and thick-walled, 0.235 long by 0.114 wide. Prostatic vesicle consisting of a short and ovoid basal portion enclosed in thickened extension of sinus sac, and a tubular and spirally coiled part which expands anteriorly to form a rounded anterior end (Fig. 15). Hermaphroditic duct muscular, about 0.152 long, slightly sigmoid, extending to genital pore. Genital pore round, small, muscular, ventral to esophagus.

Ovary rounded, 0.161 long by 0.147 wide, immediately posterior and slightly lateral to right testis. Vitellaria median to ovary, seven elongate lobes 0.168 to 0.235 long and about ⅓ as wide, joined at bases and spreading in various directions. Uterus descends to base of ecsoma; metraterm straight, beginning at level of anterior margin of acetabulum, entering base of sinus sac, extending alongside prostatic vesicle, and into hermaphroditic duct directly anterior to prostatic vesicle. Eggs yellowish, 18 to 21 by 10 to 16 (both collapsed and non-collapsed eggs).

Excretory vesicle sinuous, extending more or less medianly to posterior edge of acetabulum where it divides; crura extend forward and seem to meet dorsal to oral sucker.

Discussion: *L. spiravesiculatum* can be separated from all other species of *Lecithochirium* by the coiled posterior part of the prostatic vesicle, a character easily observed even with low magnification. The thick muscular wall of the sinus sac, the enclosure of the posterior part of the prostatic vesicle with the wall of the sinus sac, and the muscular walls of the two anterior parts of the seminal vesicle are likewise unreported in the genus. These latter characters appear in a species of *Sterrhurus* from *Cirrhitus alternatus* described below.

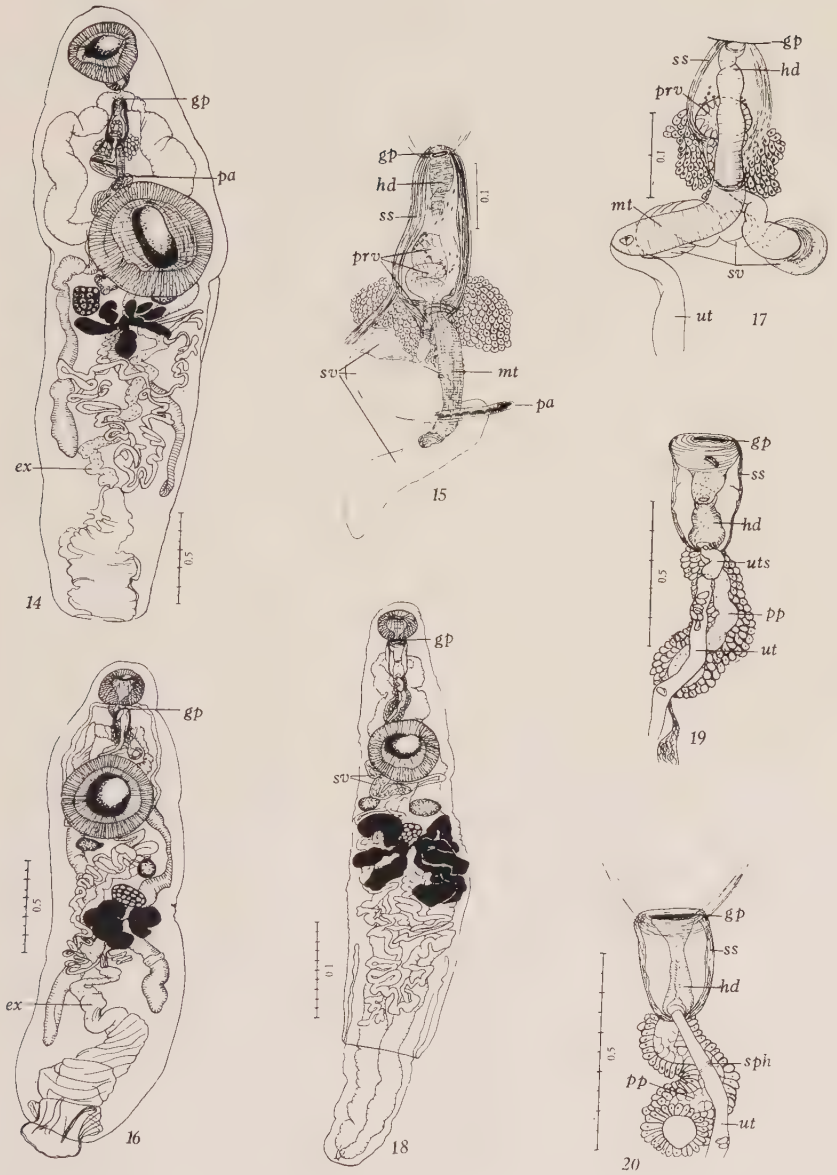


PLATE III

Fig. 14. *Lecithochirium spiravesiculatum* from *Gymnothorax undulatus*, holotype; ventral view.

Fig. 15. *L. spiravesiculatum*, holotype; ventral view of terminal genital ducts.

Fig. 16. *Sterrhurus cirrhiti* from *Cirrhitus alternatus*, holotype; ventral view.

Fig. 17. *S. cirrhiti*, holotype; ventral view of terminal genital ducts.

Fig. 18. *Tubulovesicula angusticauda* (Nicoll, 1915) Yam., 1934, from *Conger cinereus;* ventral view.

Fig. 19. *T. angusticauda* from *C. cinereus;* ventral view of terminal genital ducts showing uterine swelling.

Fig. 20. *T. angusticauda* from *C. cinereus;* ventral view of terminal genital ducts showing uterine sphincter muscle.

*Sterrhurus cirrhiti*, n. sp. (Figs. 16-17)

Host: *Cirrhitus alternatus* Gill, po'o-paa or o'opu-kai (Cirrhitidae, hawkfishes); 1 specimen from 1 of 4 hosts.

Location: Digestive tract.

Holotype: U. S. Nat. Mus. Helminth. Coll., No. 39171.

Description: Body proper 2.513 long by 0.737 wide, widest at base of ecsoma and only slightly narrower at level of acetabulum; anterior end tapered and rounded, posterior end rounded. Only a short, broad portion of ecsoma with longitudinal folds appears to be protrusible (Fig. 16); anterior portion of ecsoma sinuous with numerous, deep, transverse folds, connecting with thin-walled excretory vesicle near middle of hindbody. Numerous, minute, black granules seem to occur in tissues (rather than lumen) of both portions of ecsoma; somewhat larger granules occur in excretory vesicle. No excretory pore could be seen. Forebody muscular; parenchyma vesicular. Oral sucker subterminal, rounded, 0.221 wide by 0.191 long. Acetabulum about ¼ body length from anterior end, 0.442 wide by 0.436 long, aperture rounded. Sucker ratio 1:2. Pharynx rounded, 0.083 long by 0.087 wide, esophagus muscular, 0.072 long by 0.046 wide; ceca muscular and somewhat contracted, sinuous, ending blindly lateral to base of ecsoma.

Testes diagonal, separated by uterus; right testis immediately postacetabular, transversely elongated, 0.082 long by 0.149 wide; left testis rounded, 0.152 long by 0.112 wide. Seminal vesicle tripartite; posterior part saccular, 0.096 long by 0.056 wide, overlapping anterior edge of acetabulum; middle part small, about 0.040 in diameter; anterior part rounded, about 0.067 in diameter with very muscular wall 0.013 thick; a spacious, thin-walled, S-shaped duct about 0.096 long leads from vesicle to pars prostatica. Pars prostatica 0.056 long by 0.040 wide, lined with clear cells and enclosed by muscles of sinus sac. Prostatic vesicle round, 0.048 long by 0.059 wide, lined by vesicular cells. Hermaphroditic duct wide, extending to genital pore. Sinus sac rounded, 0.120 long by 0.080 wide with very muscular wall; posteriorly, muscles enclose pars prostatica and admit metraterm. Prostatic gland cells dorsal and lateral to posterior part of sinus sac, well developed. Genital pore a transverse slit ventral to pharynx.

Ovary transversely oval, 0.120 long by 0.181 wide, posterior to left testis, median edge lying on median line. Vitellaria immediately postovarian, right group with 4 lobes and left one with 3, lobes no longer than wide. Uterus descends medianly a short distance behind vitellaria, ascends to right of ovary, and crosses to left diagonally between testes; metraterm begins at anterior edge of acetabulum, follows seminal vesicle ventrally to median line, and turns anteriorly. Eggs yellowish, 16 to 21 by 10 to 12.

Excretory vesicle sinuous, more or less on median line, dividing at posterior edge of acetabulum, crura extending anteriorly lateral to ceca and joining dorsal to pharynx.

Discussion: This species is most like *S. lotellae* Manter, 1954 from the southern hake of New Zealand, resembling it particularly in the presence of a thick-walled anterior portion of the seminal vesicle and the rounded, muscular sinus sac. It differs, however, in that the oral sucker is smaller, the genital pore is somewhat more anterior, the pars prostatica is enclosed by the muscle fibers of the sinus sac, the eggs are slightly wider, the vitelline lobes are shorter, the acetabulum is slightly more anterior, and the ceca do not enter the ecsoma. No metraterm was mentioned for *S. lotellae*.

*Tubulovesicula angusticauda* (Nicoll, 1915) Yam., 1934 (Figs. 18-20)

HOST: *Conger cinereus* Ruppell, white eel or puhi-uha (Congridae, conger eels); 2 specimens from 1 of 42 hosts. New distribution record.

LOCATION: Stomach

SPECIMEN DEPOSITED: U. S. Nat. Mus. Helminth. Coll., No. 39177.

BRIEF DESCRIPTION of Hawaiian specimens: Total length 4.891 to 6.010, width 1.005 to 1.206. Oral sucker 0.362 to 0.382 wide; acetabulum 0.610 to 0.683 wide; sucker ratio 1:1.7 to 1.8. Seminal vesicle begins immediately posterior to acetabulum; one or two bends in posterior part, but anterior half straight and narrow. Pars prostatica begins at level of anterior edge of acetabulum, surrounded by thick, uninterrupted layer of prostatic cells; joins uterus at base of sinus sac. Hermaphroditic duct large, muscular, narrowing midway and then enlarging like a funnel; posterior part of funnel bears minute papillae while anterior part has pronounced circular muscles and is homolog of genital atrium (Figs. 19-20). Vitellaria in 2 groups of 3 and 4 lobes joined by a narrow duct immediately posterior to ovary. An inconspicuous sphincter muscle occurs near anterior end of uterus; when sphincter is constricted, tube anterior to it enlarges (Fig. 19). Eggs yellowish, 34 to 43 by 18 to 24.

DISCUSSION: A genital atrium is described for this species by Yamaguti and by Manter and appears to be a generic character. In all New Zealand specimens (25) of *T. angusticauda* this atrim is short and wide and possesses strong semicircular muscles along its ventral side; the wall of the sinus sac narrows at its base. The Hawaiian specimens also show the ventral muscles, but the wall of the sinus sac continues to the wide genital pore without constriction (Figs. 19-20).

The uterine swelling in front of a sphincter muscle near the sinus sac seems to be another unreported variation in *T. angusticauda.* This condition characterized the genus *Uterovesiculurus* (syn. of *Erilepturus*). We find that the swelling is visible in about one third of the specimens from New Zealand. The sphincter muscle is inconspicuous at best and probably has been overlooked.

The Hawaiian specimens are like the New Zealand specimens in sucker ratio, extent of the pars prostatica, and the position of the seminal vesicle. They differ in egg size, except for the single specimen from *Conger conger* with which the egg size is very similar. As pointed out by Sogandares-Bernal (1959), egg size is a "rather variable" character in species of *Tubulovesicula.* In *T. lindbergi* (Layman, 1930) Yam., 1934, the egg size is reported as 18 to 42 by 12 to 29; in *T. angusticauda,* 26 to 46 by 18 to 29.

In addition to egg size, the Hawaiian specimens also resemble the specimen from *Conger* in New Zealand in that the male duct is straight dorsal to the acetabulum, whereas in specimens from other New Zealand hosts this tube is sinuous. These differences are not considered specific, but it is notable that the Hawaiian specimens are most similar to the specimen from the conger eel in New Zealand.

Sogandares-Bernal recognized only 4 species of *Tubulovesicula*: *T. angusticauda; T. lindbergi; T. magnacetabulum* Yam., 1939; *T. pinguis* (Linton, 1940) Manter, 1947. The latter 2 species have been reported only from the type localities; *T. magnacetabulum* from a single host in Japan, *T. pingus* from a variety of hosts at Woods Hole. *T. lindbergi* and its numerous syno-

nyms have been reported from various hosts in the American Pacific, Japan, the Red Sea, the Atlantic off Morocco, and the American Atlantic off Panama. *T. angusticauda* is, so far, Pacific in distribution being reported from Australia, New Zealand, the Celebes, and Hawaii.

## Summary

Described are one new genus, *Duosphincter,* and 6 new species: *D. zancli, Aponurus acanthuri, Genolinea lobata, Lecithochirium chingi, L. spiravesiculatum, Sterrhurus cirrhiti.*

The following synonyms are proposed: *Parectenurus* Manter, 1947, syn. of *Ectenurus; Uterovesiculurus* Skrj. & Gusch., 1954, syn. of *Erilepturus; Lecithaster sayori* Yam., 1938, syn. of *L. stellatus* Looss, 1907.

The following new combinations are proposed: *Ectenurus americanus* (Manter, 1947) for *Magnacetabulum americanum; Ectenurus leiognathi* (Yam., 1953) for *M. leiognathi; Erilepturus platycephali* (Yam., 1934), for *Ectenurus platycephali.*

New host records are reported for *Hysterolecitha tinkeri* and *Lecithochirium microstomum.*

Four speies are reported for the first time from Hawaii: *Ectenurus lepidus; Lecithaster stellatus; Lecithochirium magnaporum; Tubulovesicula angusticauda.* New host records are reported for all except *T. angusticauda.*

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## Two New Species of the Genus *Paruterina* Fuhrmann, 1906, from Passeriform birds in Southern Rhodesia.

DAVID F. METTRICK*

During a survey of the helminth parasites of Rhodesian birds, two species of the genus *Paruterina* Fuhrmann, 1906 (Paruterininae Fuhrmann, 1907: Dilepididae Railliet & Henry, 1909), were recovered, which appear to be new.

*Paruterina zambiensis*, n. sp.

(All measurements in millimeters unless stated otherwise).

DESCRIPTION: Small worms, maximum length 27, maximum width 0.64. Diameter of scolex 0.31-0.35; retractile rostellum 0.08 wide by 0.05 when extended; armed with a double row of 48 hooks 18.5-19.5 microns long. Four suckers 0.14-0.15 wide by 0.12-0.13. Mature segments slightly longer than wide; typical one 0.39 wide by 0.41. Gravid segments wider than long; typical one 0.62 wide by 0.53.

Osmoregulatory system of usual pattern. Ventral canals 9-10 microns in diameter; dorsals 2-3 microns in diameter.

Genital pores alternate irregularly, open on a protuberance in middle of lateral margin of each segment.

Testes 6-10 in number, 0.044-0.052 in diameter, postovarian, and slightly lateral to ovary. Cirrus sac, 0.079-0.084 long by 0.026-0.028, runs forward from genital atrium, contains an unarmed cirrus and a slightly coiled vesicula seminalis. Vas deferens coiled, narrow, in anterior part of segment.

Vagina straight, thin walled, 14 microns in diameter, opens into genital atrium posterior to cirrus sac. Small spherical receptaculum seminis. Ovary bilobed in posterior half of segment. Vitelline gland compact, postovarian and ventral in position. Uterus appears first as a small spherical sac between ovarian lobes and just anterior to them. Later assumes shape of inverted U. Paruterine tissue develops anterior to gravid uterus.

No fully gravid segments seen. Eggs 24-28 microns in diameter.

HOST: Black Cuckoo-Shrike, *Campephaga phoenica* Latham.

LOCATION: Intestine.

LOCALITY: Zambesi Valley.

Type to be deposited in the British Museum (Natural History), London.

DISCUSSION: Unfortunately the material examined was not fully gravid, so that it is impossible to state definitely that the eggs pass into the paruterine organ.

It is proposed to place this new form in the genus *Paruterina*, because of the obvious affinities to species already in that genus. According to Daly (1958) there are 19 species in this genus, but Yamaguti (1959) lists a further five species, namely *P. iduncula* Spasski, 1946, *P. rauschi* Freeman, 1957 and *P. garrulae*, *P. kirghisica* and *P. skrjabini* all ascribed to Matevosyan, 1950. The reference given by Yamaguti to Matevosyan is in fact a summary of a doctorate thesis (apparently not published), and no mention of these species appears in the appropriate volumes of Helminthological Abstracts or Zoological Record. Also compared with Daly's list of species Yamaguti omits *P. purpurata* (Dujardin, 1845), and adds *P. melierax* which was transferred to the genus *Cladotaenia* by Fuhrmann and Baer in 1943 (Not 1944 as given by Yamaguti). Thus, there are 21 species in this genus described previous

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to the two herein described. *P. zambiensis* is separated from *P. angustata, P. guineensis,* and *P. southwelli* by having irregularly alternating genital pores. It is distinguished from the other 18 species primarily by the size, shape and number of the hooks, the number and size of the testes, size of the cirrus sac, and arrangement of the paruterine organ, and particularly from *P. candelabraria,* 40-46 hooks, 54 microns and 35-37 microns long, 24 testes; *P. chlorurae,* 40-42 hooks, 16 microns and 20 microns long, 10-12 testes; *P. cholodkowski,* 50-60 hooks, 16-18 microns long, 16-18 testes; *P. javanica,* 44-48 hooks, 25-28 microns long, 8-10 testes; *P. morgani,* 34-36 hooks, 40 microns and 66 microns long, 15-18 testes; *P. otidis,* 42 hooks, 57 and 41 microns long, 15 testes; *P. reynoldsi,* 44-48 hooks, 33 microns and 21 microns long,

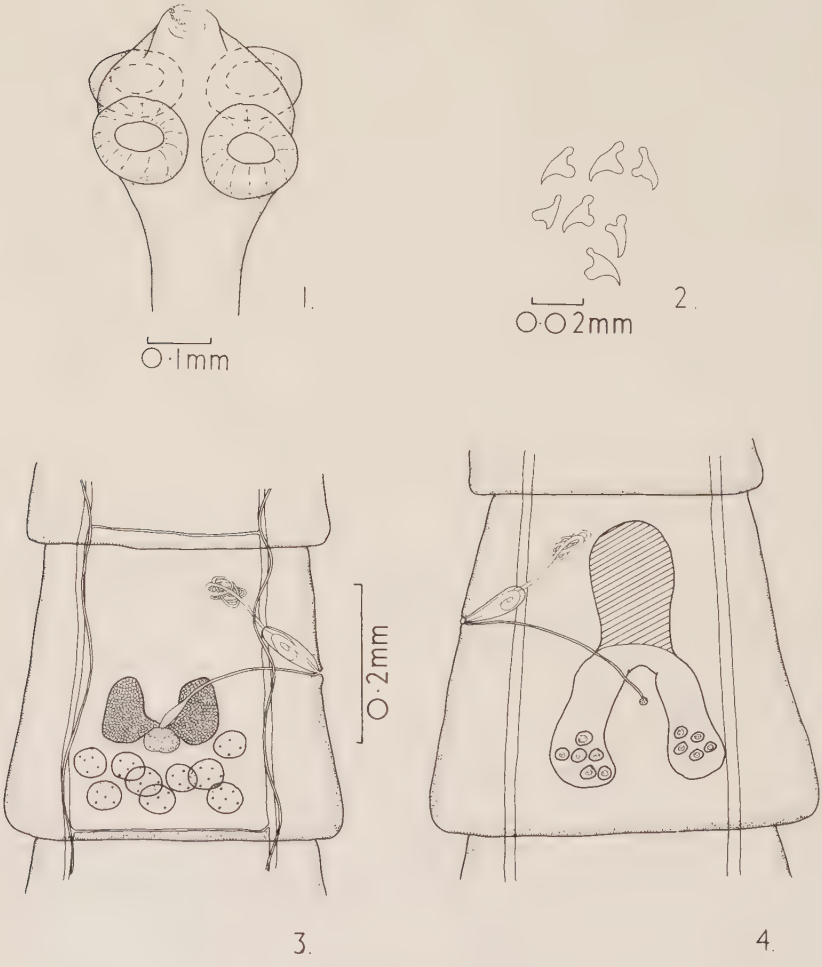


Plate I. *Paruterina zambiensis,* n. sp.

Fig. 1. Scolex; Fig. 2. Rostellar hooks; Fig. 3. Mature segemnt; Fig. 4. Gravid segment.

12-14 testes; and *P. vesiculigera,* 50 hooks, 20-26 microns and 35-46 microns long.

*Paruterina pentamyzos,* n. sp.

(All measurements in mm. unless stated otherwise).

DESCRIPTION: Small worms, maximum length 58, maximum width 1.3. Diameter of scolex 0.447; retractile rostellum 0.256 wide, when extruded has an apical sucker-like structure 0.182 in diameter; armed with double row of 42 hooks 0.080-0.082 long. Four suckers 0.133-0.142 in diameter. Mature segments wider than long; typical one 0.62 wide by 0.38 long. Typical gravid segment 1.13 wide by 1.13 long.

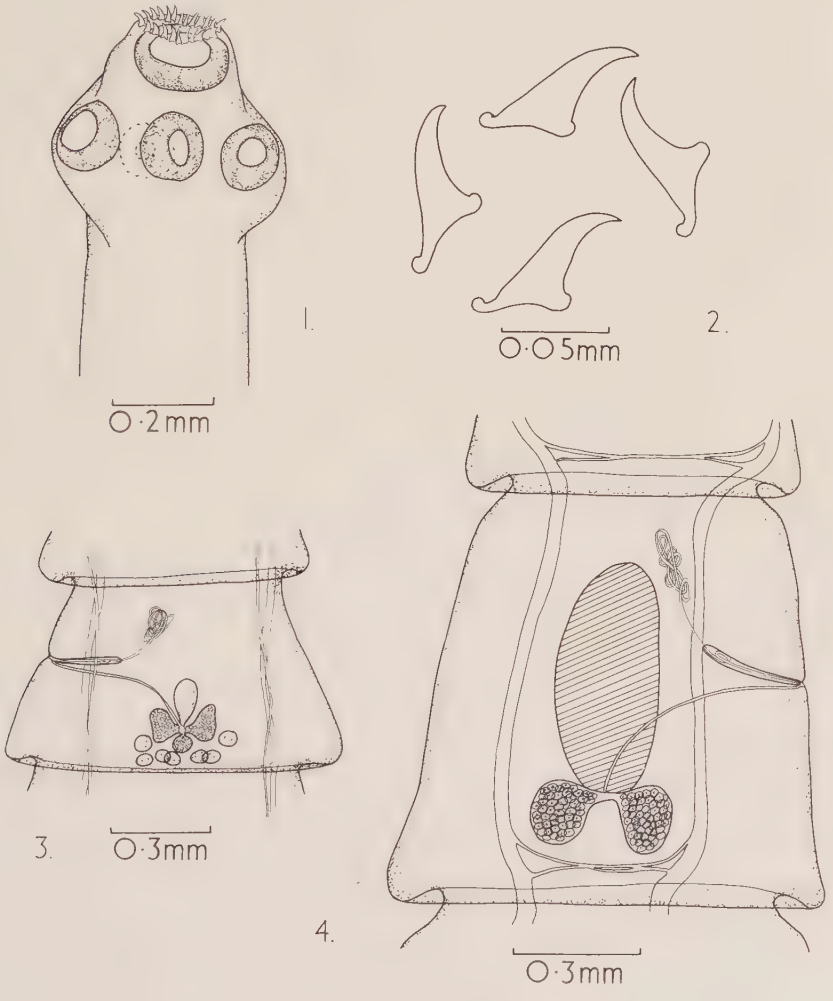


Plate II. *Paruterina pentamyzos,* n. sp.

Fig. 1. Scolex; Fig. 2. Rostellar hooks; Fig. 3. Mature segment; Fig. 4. Gravid segment.

Osmoregulatory system of usual pattern. Ventral canals 47 microns in diameter; dorsal canals 7 microns and transverse canals 11 microns in diameter.

Genital pores alternate irregularly, open marginally just anterior to the mid line, and lead into a genital atrium.

Testes 8-10 in number, 0.038-0.042 in diameter entirely postovarian. Cirrus-sac 0.196 long by 0.026 wide, runs forward from genital atrium, contains an unarmed cirrus, and a very thin coiled internal vesicula seminalis. External vesicula seminalis 0.079 long by 0.047 wide. Vas deferens, 0.011 in diameter, coiled in anterior region of segment.

Vagina 0.016 in diameter straight, thin walled. Receptaculum seminis is a dilation, 0.035 in diameter near proximal end of vagina.

Ovary bilobed in posterior half of segment. Vitelline gland 0.054 in diameter, compact, and postovarian. Mellis' gland 0.028 in diameter.

Uterus persistant; appears first as a central spherical sac, later rather horse-shoe shaped, and finaly dumb-bell shaped. Paruterine tissue develops in anterior part of segment. Eggs 0.035-0.039 in diameter. Embryonic hooks 10-11 microns long.

HOST: White Helmet Shrike, *Prinops plumata paliocephala* Shaw.

LOCATION: Intestine.

LOCALITY: Salisbury area.

Type to be deposited in the British Museum (Natural History), London.

DISCUSSION: Although provisionally placed in the genus *Paruterina,* it is possible that this species should properly fall in a new genus. The eggs are fully formed, and the embryonic hooks present but it is possible that the material was not fully gravid, and that the uterus would finally break down and the eggs pass into the paruterine organ. Further, although according to the generic diagnosis the uterus should have the form of an inverted U in the material described above the uterus has a dumb-bell appearance, having passed through the inverted U form. Pending the examination of further gravid material it is proposed that this new species be placed in the genus *Paruterina* with which it has obvious affinities.

It may be distinguished from all present species in this genus by the number, shape and size of the rostellar hooks (see discussion on *P. zambiensis*). In addition the number and size of the testes, and the arrangement of the paruterine organ and the uterus may be considered.

Both these species are the first representatives of the genus to be described from their respective hosts.

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# Studies on cysticercoid histology. II. Observations on the fully developed cysticercoid of *Hymenolepis nana* (Cestoda: Cyclophyllidea).*

MARIETTA VOGE and DONALD HEYNEMAN

This report is the second in a series of papers on the histologic organization of tapeworm cysticercoids designed to determine the relationship of the larval structures in different species.

## MATERIALS AND METHODS

Cysticercoids of *Hymenolepis nana* were grown in the confused flour beetle, *Tribolium confusum,* which was maintained on enriched flour at 30°C. All observations reported here are based on cysticercoids from beetles dissected in normal saline one and one half months after they were infected. Live material was studied under phase and light microscopes. Cysticercoids were fixed in Bouin's or Zenker's fixative. Sections from paraffin-embedded material were cut at 7 or 10 microns. Stains used were Mallory's aniline blue collagen stain (Gridley 1953), Mayer's hemalum and Van Gieson's collagen stain (Lillie 1954), Davenport's protargol (Davenport, *et al,* 1939), and Gomori's trichrome as used by Voge (1960).

## DESCRIPTION

The histologic organization of the cysticercoid of *Hymenolepis nana* is presented in figure 1, which shows a longitudinal section of the organism. The outermost layer, or external membrane, is thin and delicate and surrounds the whole cysticercoid. Beneath the external membrane there is a layer of circular fibers surrounding the body of the cysticercoid (figs. 1, and 2a). Beneath the circular fibers is the fibrous layer which consists of longitudinal fibers and corresponds to the fibrous layer in the cysticercoid of *H. diminuta* (Voge, 1960). Both the circular and the longitudinal fibers stain an intense blue with Mallory's aniline blue stain, and pink to red with Van Gieson's stain. The difference in orientation of the fibers in these two layers is shown in figures 2a, b, and d. The arrangement of these fibers can be clearly observed in living material by pressing the scolex out of the surrounding tissues and examining them under high power of the light microscope. Stained sections show no evidence of nuclei in the area of the circular fibers. However, the inner portion of the longitudinal fibrous layer contains many nuclei which stain red with Mallory's aniline blue or with Gomori's trichrome, and which can also be studied in silver stain preparations. Longitudinal sections through this fibrous layer reveal the presence of spindle-shaped and star-shaped cells (Fig. 2, c) bearing fine, and in some instances very long protoplasmic extensions which may be seen to connect with similar extensions from other cells. In this manner, a more or less intricate network is formed. In appearance,

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Aided by a grant (E-1583) from the National Institutes of Health, United States Public Health Service, Bethesda, Maryland, and a contract between the Office of Naval Research and the University of California (Nonr 233 (56.)

The technical assistance of Mrs. Nora Liu, Department of Infectious Diseases, University of California, Los Angeles, is gratefully acknowledged.

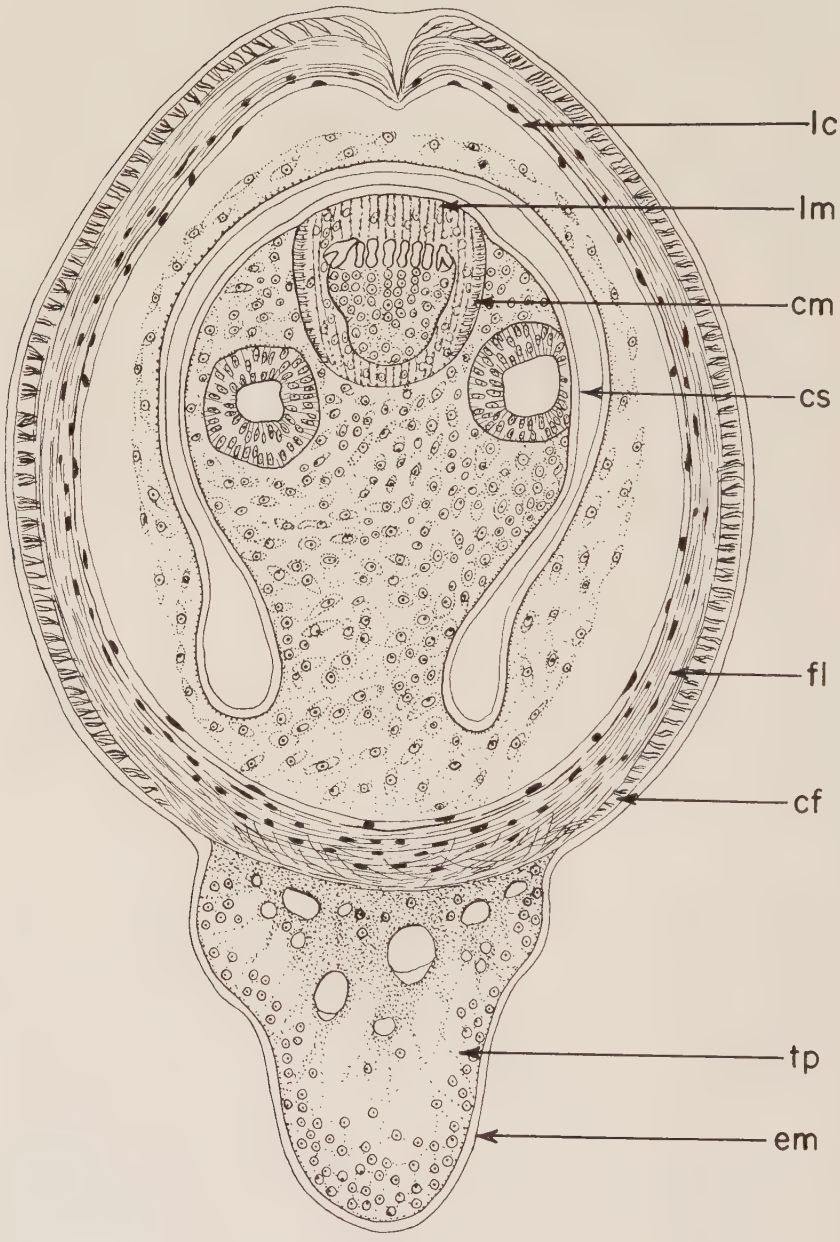


Figure 1. Free-hand drawing of the fully developed cysticercoid of *Hymenolepis nana* as observed in logitudinal sections: cf, circular fibers; cm, circular muscles of rostellar sac; cs, cuticle of scolex; em, external membrane; fl, fibrous layer; lc, lining of cysticercoid cavity; lm, longitudinal muscles of rostellar sac; tp, tail parenchyma.

these cells resemble mesenchymal cells described for adult *Fasciola hepatica* (see Hyman, 195, Fig. 85), and fibroblasts from vertebrate tissue. While the circular fibers are confined to the body of the cysticercoid, some of the longitudinal fibers appear to extend into the tail, as will be described below.

Beneath the fibrous layer is a thin layer of elongate cells referred to as the lining of the cysticercoid cavity (Fig. 1 and 2, b, d). The equivalent of this layer also occurs in *H. diminuta* (Voge, 1960); the staining reaction is similar in both species. Within the cysticercoid cavity is the scolex with surrounding tissues. These tissues, which are a continuation of the scolex proper, are in contact with the living layer or wall of the cysticercoid cavity in living material. In sectioned material, however, there may be considerable shrinkage, so that a space is frequently seen between the wall of the cavity and the tissue surrounding the scolex (Fig. 1).

The scolex is bordered by the cuticle which continues along the inside of the tissue around the scolex (Fig. 1), and consists of densely packed elongate cells. The prominent rostellar sack has two layers of muscle, an outer circular layer oriented horizontally, and an inner longitudinal layer. Both layers may be seen in living as well as in sectioned material. The rostellum, which bears the hooks, consists of densely packed cells but apparently contains no muscle fibers. In living material, one can see that the tips of the hook blades are connected by a fine thread, or perhaps a membrane, which may serve to maintain rigidity of the crown as a whole.

In living material one can observe two prominent accumulations of large granular bodies located laterally in the anterior portion of the cavity. These granules disappear upon fixation with acid fixatives; they are preserved in formalin-fixed material but are usually lost during sectioning and staining.

The cysticercoid tail consists of two distinct areas: the terminal and peripheral portions consisting of an irregular network with deeply staining nuclei and referred to as the tail parenchyma (Fig. 1), and a central area which

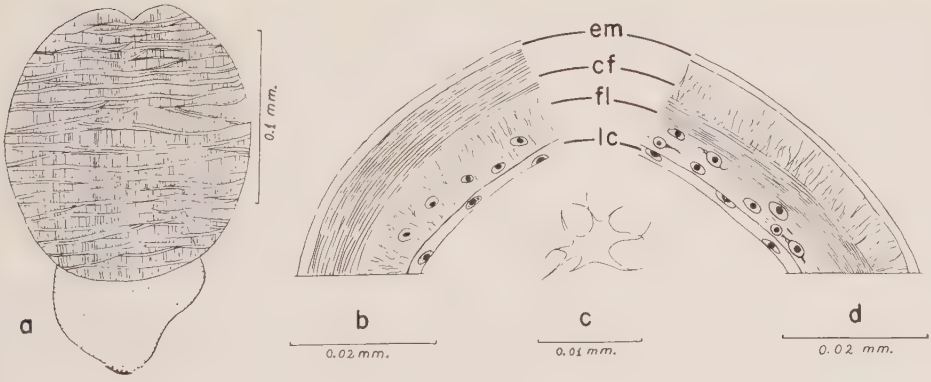


Figure 2. Camera lucida drawings. a. Surface view of cysticercoid, showing orientation of circular fibers and the longitudinal fibrous layer beneath; b. Cross-section of tissues external to cysticercoid cavity; legend as in figure 1; c. Star-shaped cell from section through inner portion of fibrous layer; d. Longitudinal section of tissues external to cysticercoid cavity; legend as in figure 1.

stains rather uniformly and heavily, containing few or no nuclei and many vacuole-like structures of different sizes. In occasional sections, this central area stains blue with Mallory's aniline blue, suggesting the presence of fibrous elements. Rarely a direct connection between the longitudinal fibrous layer in the body of the cysticercoid and the central portion of the tail was observed. However, individual fibrous elements could not be distinguished in the tail proper. The central portion of the tail stains deeply and relatively uniformly, regardless of the stain employed.

Studies on the origin and differentiation of the various layers and structures seen in the fully developed cysticercoid are in progress.

## Discussion

The histological organization of the cysticercoid of *Hymenolepis diminuta* (Voge, 1960) has been shown to be complex, particularly in the area external to the cysticercoid cavity. The cysticercoid of *H. nana* also consists of several well differentiated tissues, some of which appear to be the equivalent of those observed in *H. diminuta*. The appearance of the external membrane is similar in both *H. diminuta* and *H. nana*. However, the circular fibers beneath the external membrane in *H. nana* are very different from the hairy processes in *H. diminuta*. Studies on the origin of both structures are necessary to determine what relationship may exist between them. The hairy processes and the prominent intermediate layer in *H. diminuta* have no counterpart in the fully developed cysticercoid of *H. nana*. In the latter, the longitudinal fibrous layer, corresponding to the fibrous layer in *H. diminuta*, is situated beneath the circular fibers. This fibrous layer is adjacent to the cysticercoid cavity lining; the two structures are very similar in both species. The position and structure of the scolex and surrounding tissues inside the cavity are also comparable. One might speculate that in *H. nana* the intermediate layer, which may have been present originally, disappeared as this species became capable of undergoing a relatively rapid growth in the mammalian host.

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## The Black-head Disease of Bananas (*Musa acuminata*)

CLIVE A. LOOS and SARAH B. LOOS*

Cobb (1893) found males of the nematode, *Tylenchus similis* (a synonym of *Radopholus similis* (Cobb, 1893) Thorne, 1949) in soil around diseased banana roots in Fiji. In 1915 he described both sexes and recorded the pest from banana rhizomes in Jamaica, W.I. and sugar cane roots in the Hawaiian Islands. Leach (1958) recorded the pest on Lacatan bananas in Jamaica, W.I. Scarseth and Sharvelle (1950) reported on "head rot" and "short roots" of bananas in Honduras, two maladies they attributed to nematode injury, though the nematode was not identified. Taylor and Loegering (1953) found *Pratylenchus musicola* (Cobb, 1919) Filipjev, 1936 in frequent association with root lesions of abaca or Manila hemp (*Musa textilis* Née); *R. similis* was found but less commonly. Anon. (1957) and Loos (1957) described the symptoms associated with *R. similis* infection of Gros Michel and Cocos banana roots and rhizomes. Loos (1959) demonstrated, in pot experiments, that severe infection with this nematode, while causing a highly significant reduction in the root system of the banana, shortened the period between inoculaof young Gros Michel plants with the fungus, *Fusarium oxysporum* f. *cubense*, and symptom expression of fusarium wilt disease. Loos and Loos (1960) reported that the inadequate anchorage afforded by a depleted and severely lesioned root system, the result of *R. similis* infection, was responsible for tipping over and consequent loss in crop of first ratoon banana plants. Stover and Fielding (1958), in a limited survey of banana areas in Honduras, recorded the nematodes found in association with *Musa* spp.

Diseases caused by *R. similis* infections are better known as "spreading decline of citrus" in Florida, U.S.A. and "yellows disease of pepper" in Banka, Indonesia. "Black-head disease of bananas," suggested by Ashby (1915), appropriately describes the symptoms in banana heads or rhizomes.

Banana plants are generally spaced 11 ft. by 11 ft. apart but, in recent years on commercial plantations, closer spacings for higher plant populations have been attempted. Bananas are herbaceous perennials with a sympodial rhizome system in which horizontal growth of the sympodium is slight. This tendency for rhizome sprouts to turn up to form new aerial stems close to the parent plant results in a clumped "mat" or congregate of plants. Generally two plants are encouraged to a mat, the others being pruned back occasionally.

Primary roots are fleshy, up to two-thirds of one inch thick, and originate usually in groups of four at the junction between the central cylinder and cortex of the rhizome. These roots are restricted mainly to the upper foot of soil and may reach 17 feet in length (Simmonds, 1959). Fleshy lateral or secondary roots, of smaller diameter than primaries, are formed mainly at their distal portions (Summerville, 1939) and at damaged roots. Large numbers of fine rootlets, seldom over two inches long, which have a relatively short life and are continuously replaced, are produced on these thick fleshy roots. They originate at the junction between the tough central stele and cortex and pass through the width of the root cortex before being externally borne.

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### Disease Symptoms Associated with *R. similis*

Infections in Roots: The nematode enters a fleshy primary or secondary root close behind a root tip or via a rootlet. In the later mode of entry the nematode moves through and along the rootlet to pass into the cortical tissues of the large root, immediately above the tough central stele, near the rootlet origin. The track is clearly visible as a pink-red streak (Fig. 1-G). With growth of the colony and extension of its feeding area the pink-red coloration extends parallel with the stele and laterally through the width of the fleshy cortical tissues. Brown to black lesions, with slightly sunken centers and longitudinal cracks, up to four inches long and girdling the root are common on roots of heavily infected plants (Fig. 1-E).

The characteristic internal symptoms of *R. similis* infections are best observed in lesions over one inch long if the root is split centrally and longitudinally through the infected region. Discolored tissue is restricted to the cortex and the central stele is white and healthy (Fig. 1-F). The advancing edge or perimeter of the lesion is pink-red or wine color, with the aging center brown to black. A streak of reddened tissues may extend one-half inch beyond the lesion, in close contact with and parallel to the stele. While the internal structure of the discolored cell is destroyed the cell wall remains intact for some considerable time and the normal form of the root is retained. Final breakdown of the infected cortical tissues exposes the central stele to infection with rot-causing organisms and the root beyond the lesioned area is killed. This damage stimulates production of a number of secondary roots immediately above the damaged area (Fig. 1-B). Increased root formation, may, at first, be beneficial to the plant but in time, with a build-up of the pest population these new roots in turn become infected and are destroyed.

Steiner and Buhrer (1933) described *R. similis* in the xylem tissue of a tea root. In banana roots the nematodes often lie so close to the central cylinder that their position may be interpreted erroneously in a fairly thick tangental section.

Infection generally spread from the "seed" rhizome and, since all roots originate from rhizomes, the majority of infections are confined to the vicinity of the plant bowl. This results in shortened roots, 3 inches to 2 feet long, a symptom which Scarseth and Sharvelle (1950) mentioned. These shortened roots are inadequate anchorage to the plant, which tips over easily under wind pressure or from the leverage exerted by weight of developing fruit (Fig. 1-D). The depth of the plant bowl in the soil has, however, a bearing on proneness to tip over; plants with deep-seated bowls stand up more satisfactorily than shallow-set ones.

Infections of Rhizomes: *R. similis* infections of rhizomes cause blackening of the epidermal tissues above the lesioned area. In large rhizomes this blackening may extend up to 4 inches in width and in small material, such as "sword" suckers, the entire rhizome epidermis may be involved. Epidermal blackening is most conspicuous in the Lacatan banana and it was this symptom which Ashby (1915) termed "black-head disease."

The root of a banana plant, in a large rhizome, may pass through 3 to 4 inches of rhizome cortex before being externally borne. *R. similis* enters the rhizome via a root (Fig. 1-H) or through a wound in the epidermis. Nematodes in a root infected close to a rhizome, pass along the root and into the rhizome cortex where they spread laterally and in depth to form the typical diffuse rhizome lesion. The lesion surrounding embedded roots is clearly visi-

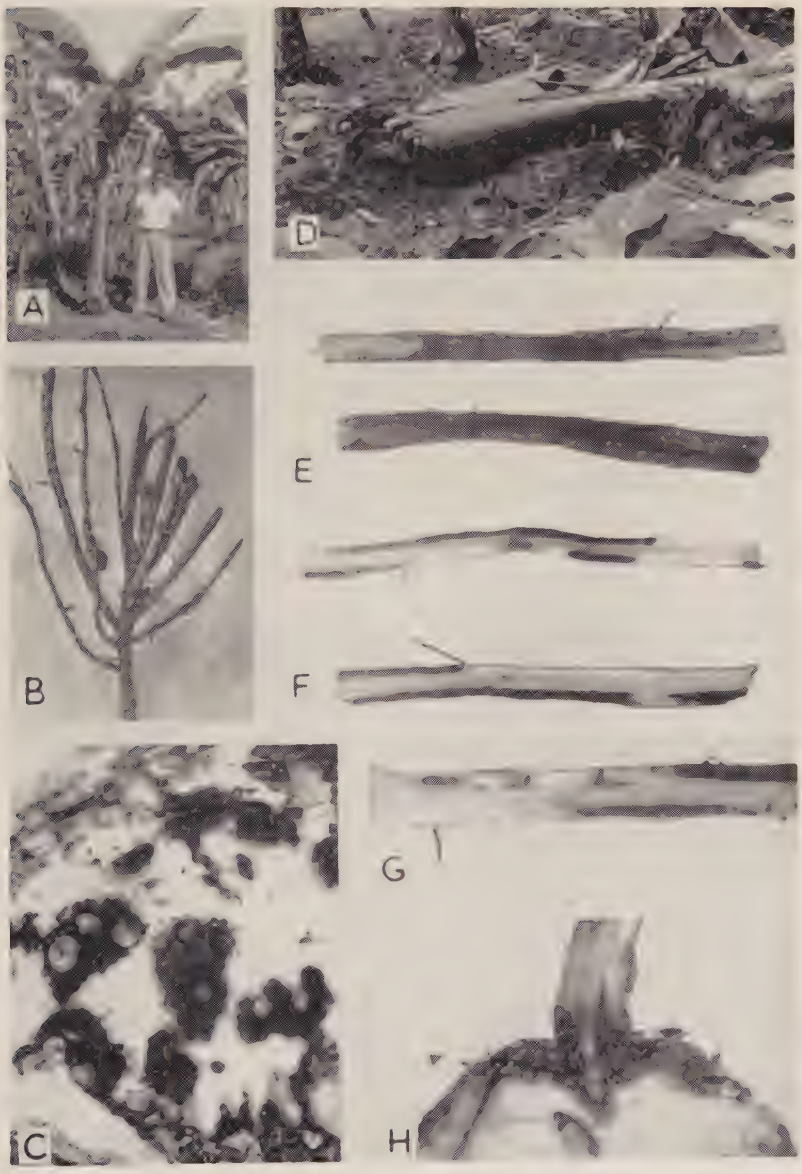


Fig. 1: A. *Radopholus similis* infected banana plant. Such plants are easily pushed over or topple over under wind pressure; B. Root proliferation above point girdled with the nematode infection; C. Rhizome lightly pared of outer cortical tissues to show diffuse R. similis lesions surrounding the root origins; D. Banana plants toppled by wind pressure. Note complete uprooting of the plant bowl; E. Lesioned primary roots of Lacatan banana; F. Split Lacatan roots showing death of cortical tissues; G. *R. similis* infection of a primary root. Note ⊥ shaped discoloration of the split root. The stem of the ⊥ is the rootlet through which the nematodes passed before turning at right angles to infect tissues parallel and close to the stele; H. Infection of rhizome cortex. The nematodes pass through the root and into the rhizome.

ble on lightly paring the outer cortical tissues of an infected rhizome (Fig. 1-C). The lesion gradually becomes brown to black in the center but edges retain the characteristic pink-red colorations. The central black tissues often disintegrate with age, forming cavities suggestive of borer (*Cosmopolites sordidus* Germar) galleries. The lesions are generally confined to the outer inch of the rhizome cortex though occasionally they may go down as deep as 2½ inches. Frequently an infection may continue below the diffused lesion as a pink-red streak inside the cortex of the embedded root.

The reddened areas are infected with all stages of the nematode whereas the blackened central tissues contain bacteria and fungi. Saprophagous nematodes become abundant in tissues which are in the process of distintegration.

All types of rhizomes, ranging from "button seed" to large "bull-heads" (Loos and Loos, 1960) are liable to infection. Dormant button buds, formed above ground level, are infected through roots which develop around their base during prolonged spells of wet weather and die back under drier conditions.

Under adverse weather conditions lesions may become water soaked and tissues prematurely invaded by rot-causing organisms. When this occurs the nematodes evacuate the lesion and the pink-red perimeter disappears.

## Location and Extraction of *R. similis*

All stages in the development of this nematode are colonized in the reddened tissues of the lesion and never beyond it. Large numbers may be obtained by teasing reddened tissues in a dish of water or by washing material, comminuted for about 20 seconds in a Waring blendor, through sieves. The specimens are found in residues on 200- and 300-mesh sieves. Larvae, juveniles and females removed in this manner are sluggish but may be induced to activity if the water, in which they are suspended, is aerated for a few hours. Males, on the other hand, are very active. Comparatively few specimens are recovered from banana soils but those in soil are generally active.

## Discussion

The behaviour of *R. similis* in different locations indicated the existence of physiological races of this nematode. The Gros Michel banana was grown, almost exclusively over the last few decades, in Almirante, Republic of Panama; infection was widespread and tip-overs abundant. During the writers' stay there a consignment of Lacatan rhizomes was imported from Jamaica, W.I., to replant an old Gros Michel area which had succumbed to fusarium wilt disease. This Lacatan material was severely infected with *R. similis,* and, as was expected, there was widespread infection throughout the planted area 12 months later. After 20 months many mats had toppled over during wind storms (Fig. 1-D) and surviving heavily infected plants could be recognized from the small stems (fruits) they bore. Those weak-looking plants could be toppled over with a slight push or pulled down with a light tug on a leaf. During a series of laboratory experiments the writers failed to obtain satisfactory infections of Gros Michel plants with *R. similis* obtained from those Lacatan rhizomes. Similarly *R. similis* from Gros Michel failed to infect Lacatan. On the other hand, using the same nematode concentrations, there was no difficulty in obtaining severe infections on Lacatan with the Lacatan strain and on Gros Michel with the Gros Michel strain. Similar specializations are evident on the banana fields in Jamaica, though definite experimental

proof is still lacking. DuCharme and Birchfield (1956) considered there were at least 3 physiological races of *R. similis* in Florida, U.S.A.

Although sandy soils may favor the spread of *R. similis* from plant to plant there were no apparent differences in the intensity of attack in heavy clay and sandy soil. This is understandable since most infections originate at the "seed" rhizome and the spread is around the plant bowl.

## SUMMARY

"Black-head disease" of bananas is caused by the burrowing nematode *R. similis*. Nematodes enter a rootlet and pass through it into the cortical tissues of the fleshy primary root where they form lesions up to 4 inches long, which may girdle the root. Lesions are brown to black with slightly sunken centers and longitudinal cracks. A root split centrally and longitudinally through the lesion shows that discoloration of the tissues is confined to the cortex; the central stele is unaffected. Edges of the lesion are pink-red while the center turns brown to black with age. Although the stele is not invaded by the nematode the breakdown of the cortex exposes it to infection with rot-causing organisms and the entire root beyond the lesioned area is killed. Root infection lies mainly in the vicinity of the plant bowl as infection usually originates from the "seed" rhizome. This results in shortened roots, a typical symptom of *R. similis* infection on bananas. The rhizome is infected via a root and the lesions are diffuse patches up to 4 inches wide and occasionally 2½ inches deep. Nematodes, in all stages of development are present in the pink-red tissues; the blackened tissues contain bacteria and fungi.

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# Morphological Anomalies in Male *Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898 (Nematoda: Trichostrongylidae) from Sheep

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Morphological anomalies have been reported in various parasitic nematodes: *Rhabditis,* (Thomas, 1941); *Haemonchus contortus,* (Madsen and Whitlock, 1958); *Marshallagia mongolica,* (Andreeva, 1958); and others. Few studies have been made, however, of the incidence of anomalies in a large number of specimens from populations of a single species. During the course of examining large numbers of *H. contortus* from sheep, the writer observed certain spicular anomalies, which appeared to occur more frequently in male worms that had been exposed to phenothiazine than in unexposed worms. This paper describes in detail some of the principal anomalies, gives their incidence in worm populations from treated and untreated sheep, and discusses their significance in relation to the taxonomy of this economically important nematode. Part of this data has been published previously in abstract (Bechlund, 1959).

## Material and Methods

The *H. contortus* populations were from 66 sheep: 21 in Georgia had had acute helminthiasis and 9 had been drenched with phenothiazine 1 to 3 weeks before necropsy; 4 lambs were at Beltsville, Md., and had been drenched with 50 grams of phenothiazine 2 weeks before necropsy; 37 sheep were from widely scattered parts of the country and *H. contortus* specimens from them had been deposited in the U.S. National Museum Helminthological Collection from 1900 to 1939 before phenothiazine was commonly used; and 4 untreated sheep were from Beltsville, Maryland and specimens from them had been deposited in the Collection from 1951 through 1958.

The *H. contortus* specimens were usually fixed in 5 percent formalin and later preserved in a solution consisting of 92 percent of 70 percent ethyl alcohol, 3 percent formalin, and 5 percent glycerin. For microscopic examination, these nematodes were cleared in a solution of 20 percent absolute alcohol and 80 percent phenol. Except in a few instances, all of the male nematodes or at least 100 from each animal were examined. The abnormal males were separated from the normal specimens at a magnification of 27X and 54X on the basis of the conformation of the spicules. Of 265 abnormal males, 40 (8 randomly selected from each of 5 treated sheep in Georgia) were studied in detail to illustrate the anomalies observed in the entire group. Several female worms in the populations containing the abnormal males were also examined for possible abnormalties.

## Results

All of the abnormalities occurred in male worms; none were observed in females. The anomalies appeared to be confined to morphological changes in the spicules, the gubernaculum, and the dorsal lobe and supporting ray of the bursa.

Description of Anomalies: The body length of the 40 anomalous males studied in detail ranged from 10 to 15 mm., whereas, normal males may be as long as 20 mm.

The spicules (Fig. II, 2-6) varied greatly in length and conformation. They were also shorter than those of the normal males which were approximately 420 microns long and equal in length (Fig. II, 1; Fig. IV). In some specimens the spicules were equal whereas in others they were unequal and differed as much as one-third of the total length. The length of the left spicule ranged from 190 to 297 microns and that of the right spicule from 178 to 330 microns. Generally, the spicules of the abnormal males were twisted along their longitudinal axis, whereas the normal spicules were straight. They also had pronounced ridges, and unlike the normal spicule terminated bluntly without small knobbed tips or had salient barblike projections near their distal ends.

The gubernaculum (Fig. I, 4) in these specimens was much shorter and broader than those of the normal male. It measured from 102 to 180 microns long by 27 to 58 microns broad, and was bluntly fusiform to "teardrop" in shape. This organ in the normal male (Fig. I, 2) measured approximately 225 microns long by 25 to 35 microns broad, and was fusiform in shape.

The lobes and supporting rays of the bursa were normal in 38 of the 40 specimens with abnormal spicules. In two males, the dorsal rays and lobes were abnormal. In one male, the branches from the stem of the dorsal ray (Fig. I, 3; Fig. III) were shorter than those of normal males (Fig. I, 1; Fig. IV), and the left branch was divided near its base to form an anomalous third long slender branch that was directed posteriorly and supported a long narrow projection of the dorsal lobe. This third branch and extension of the dorsal lobe was observed only in this one specimen. The branches of the dorsal ray in

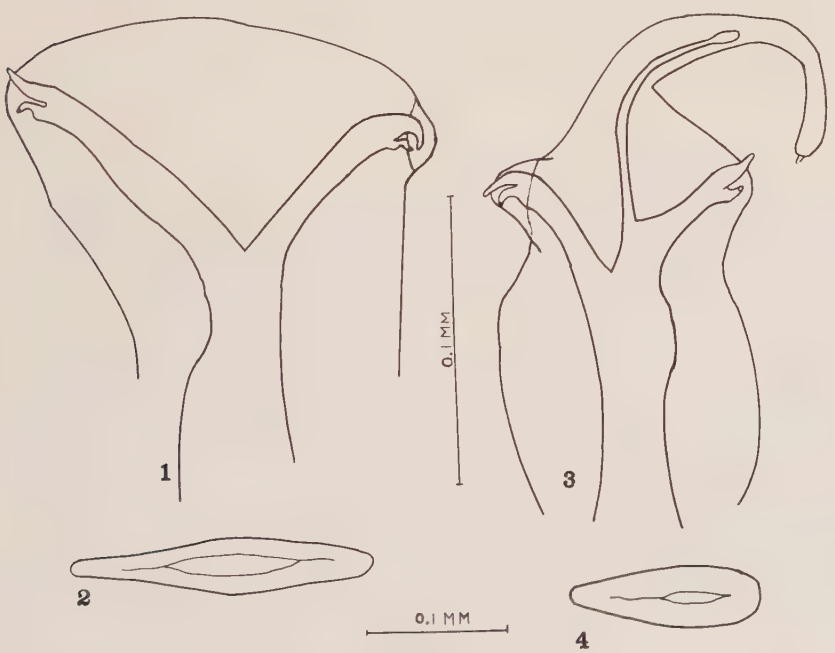


Figure I. *Haemonchus contortus* 1. Normal dorsal lobe. 2. Normal gubernaculum. 3. Abnormal dorsal lobe. 4. Abnormal gubernaculum.

the other male (not shown) were markedly unequal in length with the shorter branch deeply bifurcated at its extremity. This abnormality resulted in the formation of an asymmetrical dorsal lobe.

INCIDENCE OF SPICULAR ANOMALIES: Table I gives data on the study. The percentage of abnormal worms in 8 of the 13 exposed populations ranged from

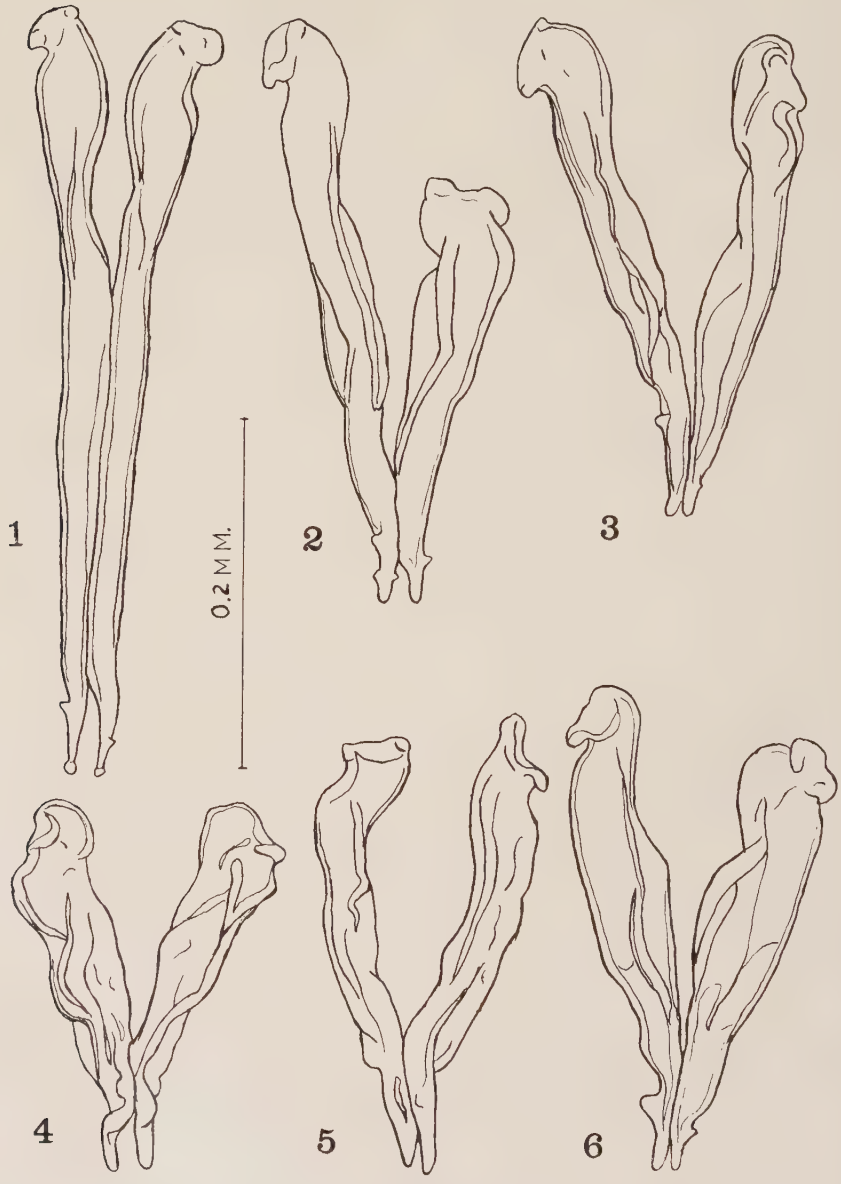


Figure II. *Haemonchus contortus.* 1. Normal spicules. 2-6. Abnormal spicules.

6.6 to 47. No abnormalities were observed in 5 exposed populations. The percentage of abnormal worms in the 53 unexposed populations ranged from 0 to 0.3.

## DISCUSSION

The results suggest that spicular anomalies occur rarely in unexposed populations of *H. contortus* but that this incidence is greatly enhanced by exposure of the worms to phenothiazine in therapeutic doses. Apparently, this anthelmintic has an adverse effect on spicular development in the larvae. The failure to observe spicular anomalies in some of the exposed populations may be due to a low incidence or absence of susceptible larvae in the host animals at the time of medication. Additional study is required to substantiate these observations.

Among the abnormal male *H. contortus* specimens were several that closely resembeled *Haemonchus lunatus* Travassos, 1914. These specimens differed slightly from the description of *H. lunatus* by having minute bifurcations at the distal ends of the dorsal ray and a more or less straight gubernaculum. *H. lunatus* was described by Travassos (1914, 1921) from an incomplete male collected from *Bos taurus* in Brazil. Lins de Almeida (1933, 1935) considered *H. lunatus* as one of 7 valid species of *Haemonchus;* however, he stated this species may prove to be an anomaly of *H. contortus.* In 1958, Madsen and Whitlock described a male *H. contortus* with deformed spicules from a sheep and referred to *H. lunatus* as a case where a comparable deformity was used to erect a new species. These writers state: "We do not wish to imply that this specimen is identical with Travassos' sole specimen of *H. lunatus,* even if the measurements agree well. The finding of such a specimen, which is quite rare in our experience, indicates that such aberrations do occur and that they are likely not to be of specific significance." In view of the foregoing observations and the fact that since the original description was published *H. lunatus* has not again been reported, it is placed in synonymy with *H. contortus,* of which it appears to be an anomaly.

Figure III. *Haemonchus contortus* male with abnormal dorsal lobe, spicules, and gubernaculum.

Figure IV. Normal *H. contortus* male.

TABLE I—Data on anomalies in male *Haemonchus contortus* specimens from treated and untreated sheep.

| Source of specimens | Sheep (number) | Male Worms (number) Recovered | (number) Examined | (percent) Abnormal males |
|---|---|---|---|---|
| | | Treated* | | |
| Georgia (1957) | 1 | 5,300 | 636 | 14.0 |
| Georgia (1958) | 1 | 8,000 | 318 | 9.7 |
| Georgia (1957) | 1 | 1,498 | 515 | 9.5 |
| Georgia (1958) | 1 | 180 | 180 | 8.3 |
| Georgia (1958) | 1 | 6,200 | 181 | 6.6 |
| Georgia (1955-1958) | 4 | 3,870 | 450† | 0 |
| Maryland (1959) | 1 | 3,840 | 100 | 47.0 |
| Maryland (1959) | 1 | 1,680 | 100 | 12.0 |
| Maryland (1959) | 1 | 1,280 | 100 | 10.0 |
| Maryland (1959) | 1 | 3,240 | 100 | 0 |
| | | Untreated** | | |
| Collection (1900-1939)# | 37 | 4,381 | 4,381 | 0.02 |
| Collection (1951-1958)# | 4 | 643 | 643 | 0.3 |
| Georgia (1955-1958) | 12 | 47,910 | 1,100† | 0 |

*Therapeutically drenched with phenothiazine 1 to 3 weeks prior to necropsy.
†Estimated.
**Never treated or had not received a therapeutic dose of phenothiazine for at least 4 weeks prior to necropsy.
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## Summary and Conclusions

Morphological anomalies involving the spicules, the gubernaculum, and the dorsal lobe and supporting ray of the bursa in male *Haemonchus contortus* specimens exposed to phenothiazine were studied.

Observations were made on 35,088 male specimens from 13 sheep treated therapeutically with phenothiazine and on 52,934 specimens from 53 untreated sheep. The percentage of worms with spicular anomalies in exposed populations ranged from 0 to 47.0; the percentage in the unexposed populations ranged from 0 to 0.3. The evidence indicates that spicular anomalies occur occasionally in unexposed *H. contortus* populations and that the incidence of these anomalies is greatly enhanced by exposure to phenothiazine in therapeutic doses.

*Haemonchus lunatus* Travassos, 1914, is placed in synonymy with *H. contortus*, of which it appears to be an anomaly.

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## *Schistocephalus thomasi*, n. sp., (Cestoda: Diphyllobothriidae) from Fish-eating Birds

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Department of Zoology, Southern Illinois University

The author spent two summers with Professor Lyell J. Thomas at the University of Michigan's Biological Station located at Douglas Lake in northern Michigan. During this time pseudophyllidean cestodes of the genus *Schistocephalus* were found as larvae in the coelomic cavities of various small fish (chiefly of the family Gasterosteidae), as adults in the intestines of fish-eating gulls and terns, and as moribund adults in the water and windrows around gull rookeries. Worms were collected and fixed in Bouin's solution. Wholemounts were stained with Delafield's hematoxylin while transverse, sagittal, and frontal serial sections cut at 10 microns were mordanted in 4 percent iron alum and stained with Heidenhain's hematoxylin. Examination of these slides of adult worms showed significant morphological differences from that described for *Schistocephalus solidus* (O. F. Muller, 1776), the only valid species in the genus. On the basis of these differences the specimens are considered to represent a new species for which the following description of the adult is given.

*Schistocephalus thomasi*, n. sp. (Figs. 1-5).

Body ovate-lanceolate; light brownish-white in color; 46 to 90 mm long; 3.8-6 mm broad. Maximum breadth in anterior ⅓ of worm. Complete segmentation of strobila with agreement of internal and external divisions. Proglottids elongate in posterior ⅓ of worm. Scolex short (0.32 mm), triangular, with apical pit; no bothria present. Neck absent. Segments without lateral emarginations. Cuticle 6 microns thick and composed of two noncellular layers. Cortical musculature in form of alternating layers. Outermost longitudinal muscle in bundles 12 microns thick; outer circular muscle bundles five microns; inner longitudinal bundles 42 to 60 microns thick and 30 microns wide; and innermost circular muscle bundle 20 microns thick. All muscle layers continuous through length of strobila. Outer two layers also continuous at lateral border from dorsal to ventral surface. Inner two layers not passing around side. Functional reproductive organs present in all proglottids beyond 16th or 17th except for undifferentiated terminal segment. Testes oval, 70 to 78 microns by 40 to 60 microns and number about 300 per segment; extending throughout dorsal medullary parenchyma except lacking above uterus and vas deferens. Vas deferens coiled and expanded up to 35 microns; convoluted above uterus; passes into seminal vesicle by single, curved duct 20 microns in diameter. Seminal vesicle far anterior; ovale (120 by 150 microns) circular to (150 microns in diameter); thin wall of 14 microns poorly muscularized, passing directly by constricted tube of 15 microns diameter into

cirrus sac. Cirrus sac circular to trigonal shape, 150 to 180 microns in diameter; located directly below seminal vesicle; loose cellular wall 18 microns thick containing scattered muscle fibers. Ductus ejaculatorius not distinguishable from cirrus.

Ovary dumbbell-like in shape 200 microns wide with lateral expansions 80 by 66 microns and 50 microns thick. Ovary ventrally located in posterior-middle of proglottid. Oviduct emerges from center of isthmus between ovarian lobes. A poorly developed oocapt 24 microns in diameter located at point of emergence; oocapt of a delicate, low cuboidal to squamous epithelium surrounded by inner circular and outer longitudinal and diagonal muscle fibers. Vagina 30 microns in diameter with a slight expansion immediately prior to its union with oviduct; unites with oviduct beneath uterine coils along midsagittal line. No seminal receptacle. Oviduct proceeds obliquely dorsolaterally to ootype, into which common vitelline duct also empties. Ootype surrounded by Mehlis' gland; the entire complex being about 50 microns in diameter.

Vitelline glands extend in an unbroken sheet from proglottid to proglottid in cortical layer between outer circular and inner longitudinal muscle bundles, completely encircling each mature segment except for areas above and below the uterine coils and around genital atrium. Individual acine numerous, 30 to 48 microns in diameter except a few laterally located up to 90 by 48 microns. A common vitelline duct 15 microns in diameter formed in median, ventral, cortical layer by union of two short right and left vitelline ducts 8 microns in diameter. Common vitelline duct proceeds dorsal directly to cotype, expanding slightly into a club-shaped vitelline reservoir 40 by 25 microns. Uterus proceeds anterio-ventrally from ootype; expanded with eggs, convoluted, and with a thin, delicate epithelium. Its terminal coil brings uterus to a position posterior and slightly to one side of cirrus where it passes through cortex to open as uterine pore in genital atrium. This pore alternates irregularly from left to right side of the cirrus pore.

The oval, operculate eggs measure from 54 to 60 microns long by 35 to 40 microns wide and undergo cleavage while retained in uterus.

Two major nerve trunks occur laterally located. Each about 60 microns in diameter with only a slight expansion in scolex where they unite.

Numerous small, thin walled excretory ducts branch and anastomose throughout the medullary parenchyma, unite in anterior and posterior ends of worm.

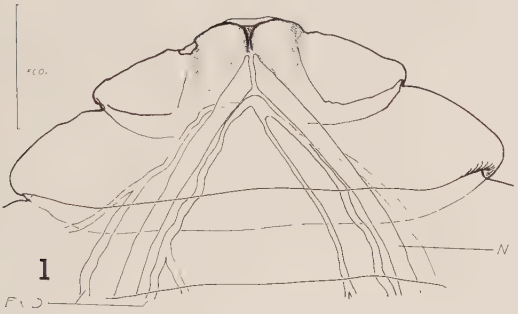
Hosts: *Larus argentatus* Pontoppidan (Herring Gull) and *Sterna hirundo* L. (*Common Tern*).

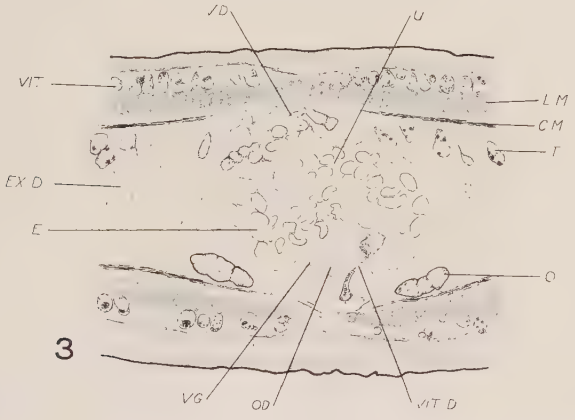
Habitat: small intestine

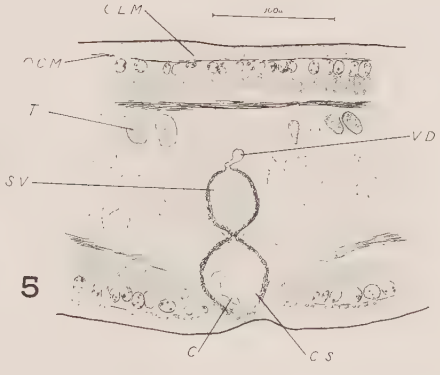
Locality: Pismire Island, Lake Michigan, Emmet County, Michigan.

Specimens: U.S.N.M. Helm. Coll. No. 39416 (holotype) and 39417 (paratype).

Distinguishing features separating *S. thomasi* from *S. solidus: Schistocephalus solidus* is described as having a large seminal receptacle; a seminal receptacle is absent in this material of *S. thomasi.* All valid descriptions of the vas deferens of *S. solidus* place the expanded, coiled portion ventrally and closely applied to seminal vesicle. This is not the condition found in these specimens. Here the coiled vas deferens is located above the uterus and proceeds ventrally by a relatively long, narrow duct into the seminal vesicle. The seminal vesicle also appears different in the two species, surrounded by a thick, muscular wall in *S. solidus* while in *S. thomasi* the vesicle wall is very thin and poorly muscularized.








All drawings made with the aid of a camera lucida.

Fig. 1. Scolex of whole mount showing nerve cords and excretory ducts.

Fig. 2. Egg showing segmentation.

Fig. 3. Cross section through mature proglottid showing most female organs and their arrangement.

Fig. 4. Cross section detail of extended cirrus.

Fig. 5. Cross section through anterior portion of mature proglottid showing most male organs and their arrangement.

CM, circular muscle; C, cirrus; CS, cirrus sac; E, egg; EX D, excretory duct; LM, longitudinal muscle; N, lateral nerve cord; OP, operculum; O, ovary; OD, oviduct; OCM, outer circular muscle; OLM, outer longitudinal muscle; SV, seminal vesicle; T, testes; U, uterus; VG, vagina; VD, vas deferens; VIT, vitellaria; VIT D, vitelline duct.

## DISCUSSION

The genus *Schistocephalus* appears to be cosmopolitan in distribution having been found in such scattered localities as the British Isles, Sweden, Italy, Haiti, Greenland, and the United States (Alaska, California, and Montana). However, almost all of the morphological and taxonomic studies of the genus have been made from European specimens, the latest one is by Hopkins and Smyth (1951). Arthur R. Cooper (1918) in his monograph on the Pseudophyllidea compared the available descriptions of European species and demonstrated that all of the previous specific differentiations were based upon natural variations between individuals or different stages in the life history of the single species *Schistocephalus solidus* (O. F. Muller, 1776). Cooper's study of American material, however, was limited to the five lots and among these he had only one mature specimen. From this he concluded that the American species is the same as that found in Europe. Linton, the only other American to report on the morphology of *Schistocephalus* (1898, 1927) gave very little detailed information, confining his remarks primarily to external features and measurements of the single adult recovered from a pied-billed grebe (*Podilymbus podiceps*) collected at Woods Hole, Massachusetts. He accepted the European name *S. dimorphus* Creplin, 1829 (=*S. solidus* (Muller, 1776)) with reservation since his specimen showed such distinctive external characteristics as lateral emargination of the anterior segments, the presence of two flat, leaf-like brothria, and the absence in larvae of single dorsal and ventral longitudinal median furrows.

Creplin in 1829, after a study of the genus *Bothriocephalus,* found that *B. solidus* did not belong to this group and erected a new genus and species for it, *Schistocephalus dimorphus.* Most taxonomists readily accepted the establistment of this new genus but felt that he violated the law of priority in not continuing the specific name first given by O. F. Muller (1776) for the larval stage of this worm (*Taenia solida*).

During the 18th and 19th centuries these five additional species were described: *Schistocephalus gasterostei* (Fabr., 1780), *S. nodosus* (Block, 1782), *S. dimorphus* Creplin, 1829, *S. rhynchichtlydis* Dies., 1863, and *S. zschokkei* (Fuhrm., 1896). They have all been relegated to synonymy with *S. solidus* (O. F. Muller, 1776) by subsequent work, especially that of Cooper (1918) and Joyeux and Baer (1936).

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# Notes on the Identities of Mermithid Parasites of North American Mosquitoes, and a Redescription of *Agamomermis culicis* Stiles, 1903

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At least nine reports of mermithid nematodes parasitic in mosquitoes are in the North American literature. Of these only three name and describe the parasite: the others give only a few measurements or biological details. This lack of identification stems from the fact that parasitic mermithids are larvae and possess few of the adult characters used in the identification of the Mermithidae. A study of the growth and habits of *Hydromermis churchillensis* Welch, 1960, made it possible to analyse the biological details given in some of these reports. The procurement of type material, and of specimens on which these reports were based permitted a comparison of the forms and a determination of the actual number of species involved in the nine records.

Stiles (1903) described *Agamomermis culicis* Stiles, 1903, from the two specimens collected by Smith (1903) from adult *Aedes sollicitans* (Walk.). Stabler (1952) recorded a mermithid from larvae of *Aedes vexans* (Meig.), *Culex salinarius* Coq., and *Culex pipiens* L. Comparison of Stabler's specimens with the type of *A. culicis*, U.S.N.M. Helm. Coll. No. 9401, showed them to be the same. This is also supported by the fact that Stabler's worms were collected within 60 miles of Smith's. Parasitism of different stages of the host, or different hosts, appears to be unimportant, as larvae of *H. churchillensis* were found by Welch (1960) in both larvae and adults of *Aedes communis* (DeG.), and in larvae of *Mochlonyx* sp. and *Chaoborus* sp., both genera of another subfamily. The mermithids reported by Johnson (1903) in larvae of *Anopheles* sp. from New Jersey were probably also *A. culicis*.

Hearle (1926) observed a nematode in adults of *Aedes vexans* in British Columbia in 1920 and 1921. Steiner (1924) identified this mermithid as *Paramermis canadensis* Steiner, 1924. He described six hypodermal chords in this species but illustrated eight, so that the generic assignment of the species is doubtful. The types were housed in the Canadian National Collection and found to be larvae. Examination revealed eight chords and an absence of criss-cross fibres in the cuticle, characters suggesting *Hydromermis* Corti, 1902. Definite assignment of the species to this genus is prevented, however, by the absence of definitive adult characters in these larvae, and poor condition of the specimens. It would be better for the present to designate this species a *species inquirendae* and assign it to the collective group, *Agamomermis* Stiles, 1903, for species based on immature specimens.

Jenkins and West (1954) found a mermithid parasitizing larvae of *Aedes communis* (DeG.) at Churchill, Manitoba. Welch (1960) named this nematode *H. churchillensis* and described its bionomics and incidence.

Hearle (1929) found an adult female *Aedes flavescens* (Müller) in British Columbia to be parasitized by a small nematode. It is unlikely that this was a mermithid.

Larval mermithids found in mosquito larvae and adults have little internal structure visible, their body being completely filled with an opaque trophosome. A small caudal structure, variously named the caudal appendage, the terminal spine, or the caudal papillae is present. Several authors attribute

diagnostic significance to it, but this seems unjustified since it occurs in most mermithids and represents only the terminal portion of the larval tail. After emergence from the host the internal structure of the free-living fourth larva is more easily seen, but the gonads are not yet formed. After moulting the trophosome in the adult almost disappears and the body is filled by the gonad. With care head structure can be seen in the parasitic and free-living larvae and is of diagnostic value.

My studies suggest that three species of which two are *species inquirendae* have been found parasitic in North American mosquitoes. Laird (1956) reviewed the world records and postulated the existence of at least four species. One of these included Stabler's (1952) form of *A. culicis*. Without material it is difficult to determine if any of Laird's other three species are similar to *A. canadensis* or *H. churchillensis*.

Free-living larval stages of the three species from North America closely resemble one another, but may be separated by several combinations of characters. A comparison of the ratio of head width and greatest body width shows this ratio to be one-third to one-fourth for *H. churchillensis* and *A. culicis* and one-fifth for *A. canadensis*. The amphids of the first two species are also smaller and less conspicuous than those of the last species. The head has a flatter cone-shape in *H. churchillensis* than *A. culicis*, and when the spicule anlagen is present it is shorter and more robust in the former than the long slender anlagen of the latter. The terminus within the last larval skin of *A. culicis* is rounded, that of *A. canadensis* pointed, and that of *H. churchillensis* usually irregularly rounded.

The following is a redescription of *A. culicis* based on Stiles' type and Stabler's specimens.

*Agamomermis culicis* Stiles, 1903

Immature adult within larval cuticle: Stiles', 1 specimen: L 11.1 mm., W 0.18 mm.

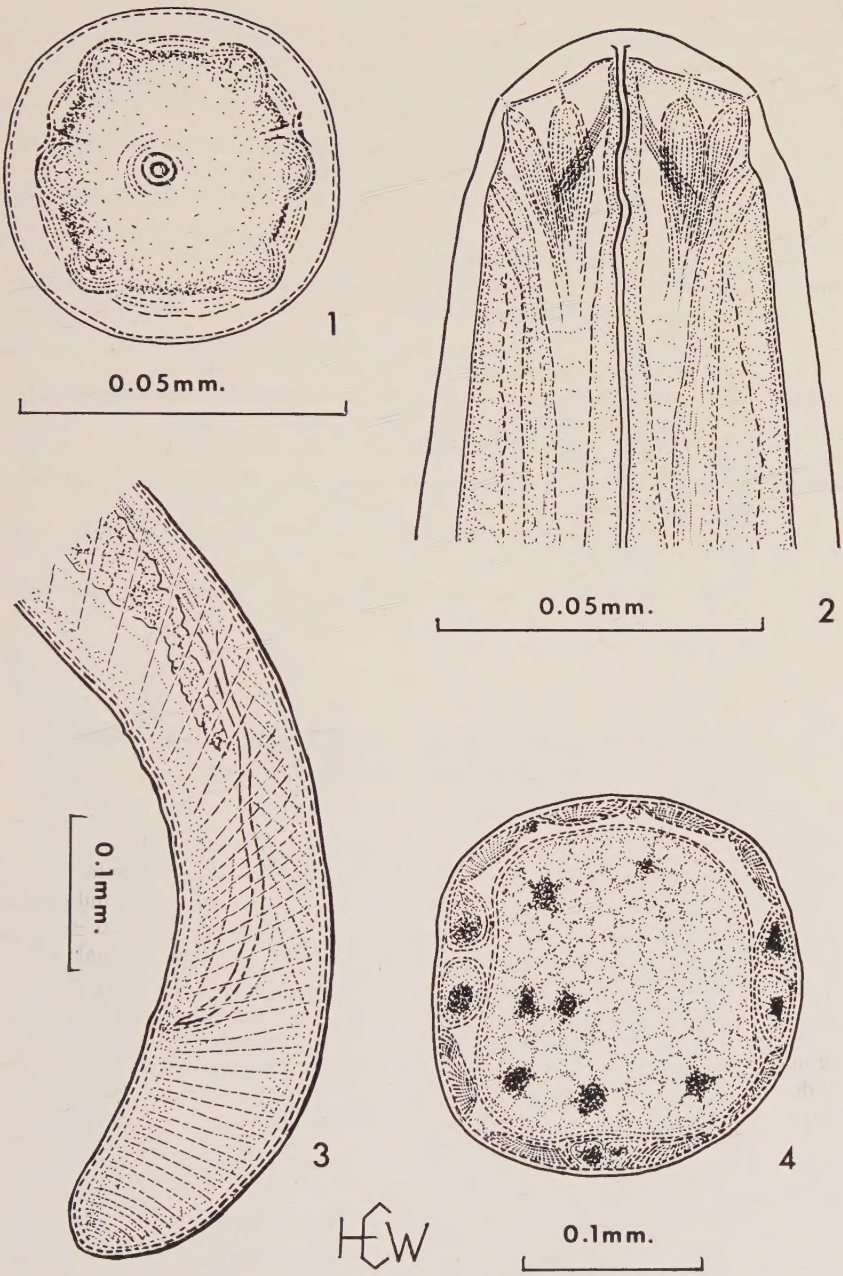
Stabler's, 9 specimens: L Av. 14.8 ± 0.6, R 11.8-17.5 mm.; W Av. 0.19 ± 0.01, R. 0.15-0.21 mm.; HW Av. 0.049 ± 0.002, R 0.043-0.056 mm.

Body tapered from nerve ring forward, and of even width behind nerve ring. Head width/maximum body width, Stiles 0.30; Stabler, Av. 0.26 ± 0.01. Cuticle thin, 2-3 microns, without criss-cross fibres. One hypodermal chord in each dorsal, ventral, lateral, and submedial position; lateral chords each contain three rows of cells. Head homocephalic; lip-region cone-shaped; groups of papillae in each lateral and submedial position, in one plane, hexagonal in position in face view. Amphids small, pouch-shaped, opening slightly dorsal to lateral papillae. Mouth terminal, oesophagus narrow, 3-4 microns, length undetermined. Nerve ring 180 microns from mouth. Trophosome commences 200 microns from mouth, terminates 240-250 microns from terminus, filled with fat globules. Adult terminus rounded, cuticle drawn out into filament 60-100 microns in length.

Immature Male: Stabler's, 1 specimen: L 9.5 mm., W 0.13 mm.

Body, cuticle, hypodermis, and head structure similar to female. Single slender spiculum, 229 microns long, 69 microns wide, slightly curved, with base 0.42 mm. from terminus. Tail tapered to rounded terminus.

The species could be assigned to *Hydromermis* as erected by Corti, 1902, but not to this genus as emended by Daday, 1911. Assignment of the species at this time would be unwise, for the female of the species is unknown and the generic diagnosis of the mermithids unsettled.




*Agamomermis culicis* Stiles, 1903

Fig. 1. Head of immature female, end on view.
Fig. 2. Head of immature female, dorsal view.
Fig. 3. Tail of immature male, lateral view.
Fig. 4. Cross section of immature female.

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## *Capillaria bonnevillei*, n. sp., (Nematoda: Trichuridae) from the Ord Kangaroo Rat in Utah*

ALBERT W. GRUNDMANN and JOHN C. FRANDSEN

One complete female (the allotype) and 4 complete male nematodes (holotype and paratypes), in addition to several incomplete worms, were recovered from formalin-preserved hosts. The nematodes were cleared in Amann's chlorolactophenol and examined in temporary lactophenol mounts. Female measurements are those of the allotype only. The sample of twenty eggs measured was drawn from several females. In the description of males, measurements and numerical data are given for the holotype first, with the corresponding data for the 3 paratypes following in parenthesis. All measurements are in millimeters.

*Capillaria bonnevillei*, n. sp.

FEMALE: 19.8 long; 0.152 wide just posterior to vulva; maximum width 0.200. Fine cuticular annulations present. Bacillary line not observed. 36 paresophageal nuclei. Beginning of stichosome 0.087 from anterior end; length of stichosome 4.640. Vulva slightly posterior to termination of esophagus, 5.8 from anterior extremity; labia slightly salient; heavily muscular ovejector present. Gravid uterus fills body cavity. Measurements of sample of 20 ova: length: range 0.041 to 0.059, sample mean 0.0511, sample standard deviation 4.8; width: range 0.020 to 0.029, sample mean 0.0243, sample standard deviation 0.6. Outer shell of egg with numerous irregular, short,

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This study was supported by the University of Utah Research Committee and by a grant (G-5280) from the National Science Foundation.

longitudinal striae; inner shell forms slight collar for opercular plugs at ends of ovum. Cauda terminates in 3 lobes, 1 terminal, 2 latero-ventral. Anus slightly subterminal, situated at base of caudal lobes. Vulva divides body 1:3.4.

MALE: 14.9 (15.1, 16.0, 12.35) long; maximum width 0.130 (0.152, 0.148, 0.139). Fine cuticular annulations present. Bacillary line not observed. 36 paresophageal nuclei present in holotype, 33 present in the 1 paratype in which they were all distinctly visible. Termination of esophagus 4.54 (4.65, 4.55, 4.26) from anterior end. Spicule sheath armed with fine spines. Spicule stout, with faint transverse striae. Spicule 0.285 (0.300, 0.275, 0.240) long, maximum width 0.020 (0.020, 0.016, 0.016). Cauda terminated by 2 poorly-formed lobes, each lobe bearing a single bilobate papilla (Fig. 4). Bursa lacking. Cloacal opening slightly subterminal, at base of caudal lobes. Termination of esophagus divides body 1:3.3 (1:3.2, 1:3.5, 1:2.9).

HOST: Ord Kangaroo Rat *Dipodomys ordii marshalli* Goldman.

HABITAT: Duodenum.

LOCALITY: Vegetated dunes of Stansbury Island, Great Salt Lake, Tooele County, Utah. Elevation 4,200 feet. Collected 11 July 1957.

REPOSITORY OF TYPES: Holotype and allotype in Helminthological Collection, United States National Museum, Washington, D. C. Paratypes (3) in Museum of Zoology, University of Utah, Salt Lake City.

In the key of Read (1949), this species keys down to *Capillaria americana* Read, 1949. The present species differs from *C. americana* by possession of

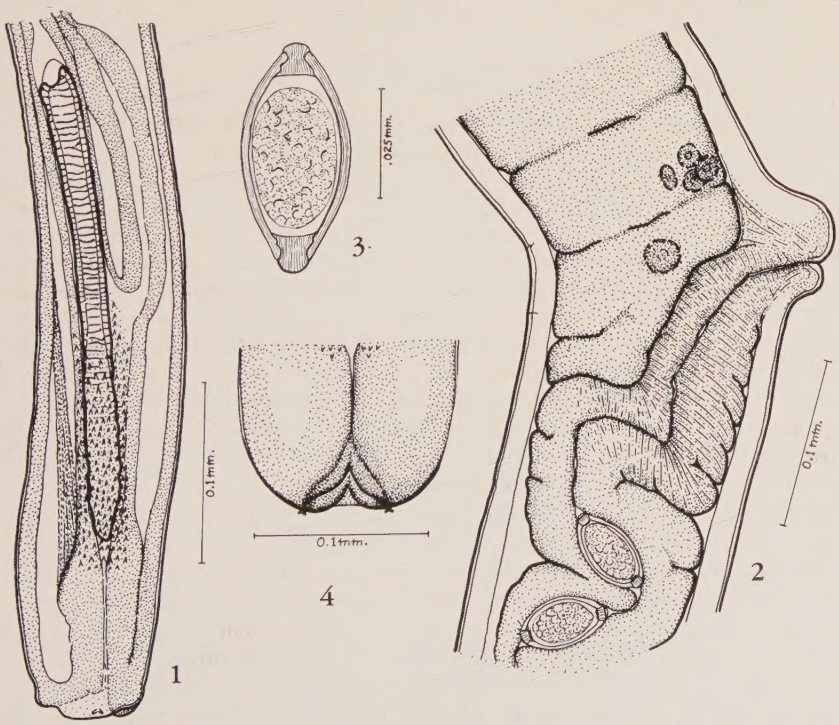


Figure 1. Posterior end of male, showing armed spicule sheath.
Figure 2. Vulvar region of female.
Figure 3. Egg.
Figure 4. Cauda of male, showing bilobate papillae.

the following morphological features: an armed, instead of an unarmed, spicule sheath; bilobate papillae on the caudal lobes of the male, in contrast with simple papillae on the caudal lobes of males of *C. americana*; and the apparent absence of bacillary lines in specimens of *C. bonnevillei*, as contrasted with their presence in specimens of *C. americana*. Members of the 2 species would also appear to differ with regard to measureable characters, but here the small size of the sample of *C. bonnevillei* handicaps one in making accurate estimates of the parameters.

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## Brayton Howard Ransom Memorial Award

Trustees of the Brayton H. Ransom Memorial Trust Fund announce the establishment of a commemorative award in recognition of excellence and achievement in parasitology.

The award will be known as the Brayton Howard Ransom Memorial Award and will be administered by the Trustees of the Fund. It will be given at irregular intervals, but not more often than annually.

Conditions of the award impose no restrictions of nationality, race or creed, or of professional or scientific affiliation.

Nomination of a candidate may be made at any time by anyone identified with parasitology and related sciences. It should be sent to the Trustees of the Fund and be accompanied by a supporting statement.

NOTE: Because of Dr. Ransom's distinguished association with the Helminthological Society of Washington and the continuing dedication of that Society to the broad parasitological interests of Dr. Ransom, the Trustees have decided to make the first award, if feasible, on the occasion of the commemoration of the fiftieth anniversary of the Helminthological Society of Washington, October 8, 1960, and on this occasion, to give some preference to a recipient whose main work is identifiable with the general lines of investigation pursued by Dr. Ransom.

Trustees of the Brayton H. Ransom Memorial Trust Fund
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## Number 3 (Special) of Volume 27

The Table of Contents for this volume, The Minutes of the Society Meetings, Report of The Brayton H. Ransom Memorial Trust Fund, and Related Material will be included in Number 3 (Special) of this volume which will be issued after the Fiftieth Anniversary Meeting to be held on Saturday, October 8, 1960.